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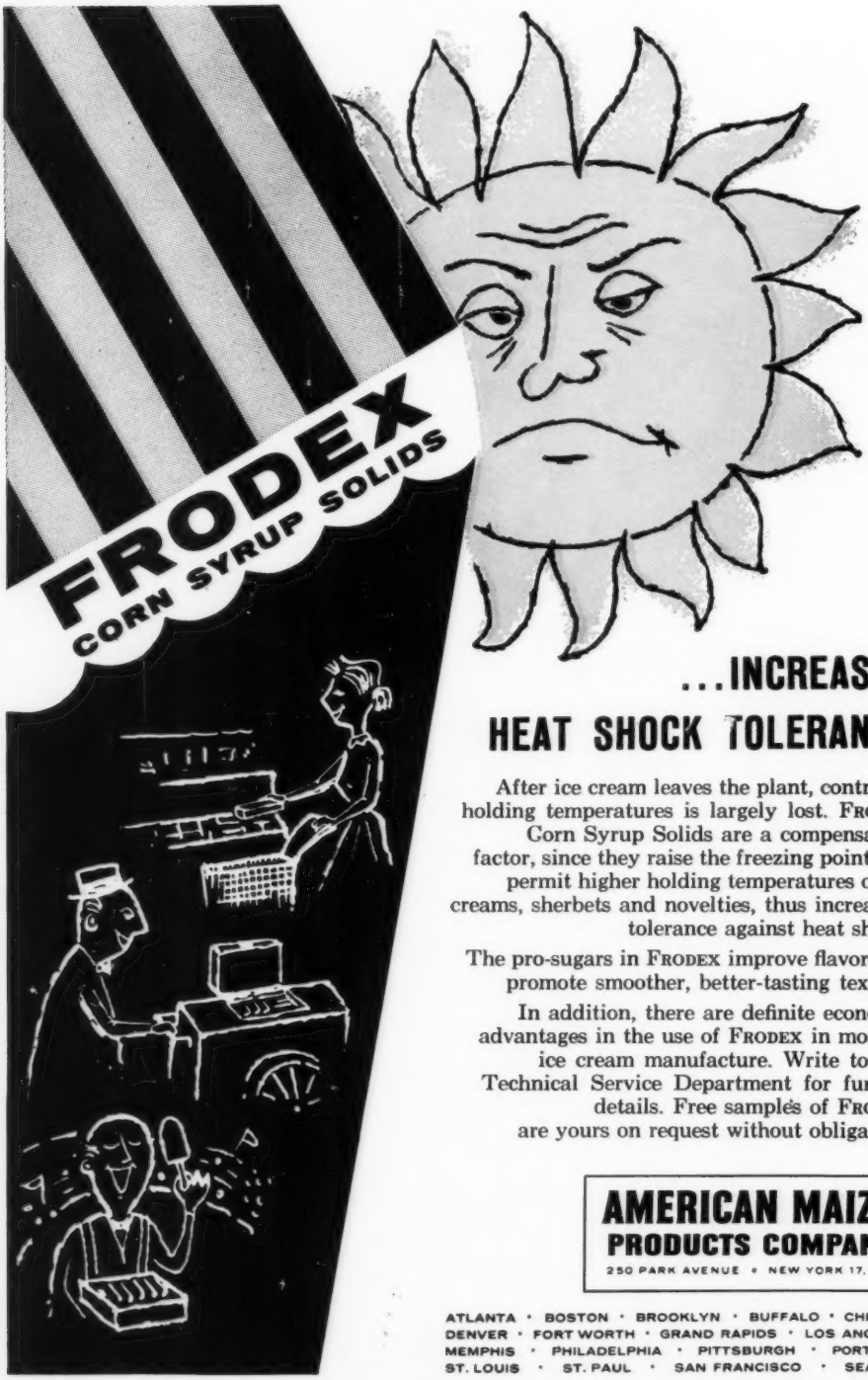

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PEOPLE AND EVENTS

A. H. Robertson New President of AOAC

A. H. ROBERTSON, New York State Dept. of Agriculture and Markets, is the newly elected president of the Association of Official Agricultural Chemists. Dr. Robertson is a prominent member of A.D.S.A.

A. H. Robertson

He is recognized as one of the leading dairy bacteriologists in the United States and is especially interested in the problems with frozen foods. Development of frozen foods, their commercial expansion, the problem of sanitation, and the future of frozen foods are especially engaging Dr. Robertson's attention. In his presidential address to the Association next year he plans to discuss various phases of the frozen food industry and venture a prophecy for the future for frozen products in American living.

He edited the ninth and tenth editions of *Standard Methods for the Examination of Dairy Products* for the American Public Health Association. He assists in the critical evaluation of analytical procedures, in making recommendations for acceptance of methods by official agencies, and in directing studies to establish the reliability of methods. Accepted methods are published in *Official Methods of Analysis* by the AOAC.

Dr. Robertson earned his Ph.D. degree at Cornell Univ. He is a veteran of World War I, former city bacteriologist of Geneva, N. Y., assistant at the New York State Agricultural Experiment Station, and bacteriologist for the Vermont Agricultural Experiment Station.

He has been associated with the New York State Food Laboratory for 30 years and since 1930 has been director. He is the author of several publications on bacteriology and chemistry of foods.

Results of the Collegiate Students' International Contest in Judging Dairy Products

The Univ. of Minnesota team won the 24th Collegiate Students' International Contest in the Judging of Dairy Products, held at Kraft Foods in Chicago. Thirty teams participated. Second and third places were won by teams from Michigan State Univ. and Univ. of Connecticut, respectively. These three teams were awarded the Dairy Industrial Fellowships, furnished by the Dairy Industries Supply Association, valued at $1,800, $1,650, and $1,500, respectively.

In addition to winning first place in judging all products, the Minnesota team placed first in the judging of milk, cheese, and butter. The team from California State Polytechnic College won first in the scoring of ice cream.

Individual first place winners in the judging of butter, cheese, milk, ice cream, and all products went to representatives from South Dakota State College, Washington State College, Michigan State Univ., Univ. of Connecticut, and the Univ. of Minnesota, respectively. Gold watches were awarded to the first place winners in each of the four products. The Minnesota team members placed first, second, and third in the judging of all products.

The contest held this year was the 37th contest in the judging of dairy products sponsored wholly, or in part, by the American Dairy Science Association and the 24th sponsored by the Dairy Industries Supply Association. The 30 teams in the 1958 contest represented the Univ. of Arizona, California State Polytechnic College, Clemson Agricultural College, Univ. of Connecticut, Cornell Univ., Univ. of Georgia, Univ. of Illinois, Iowa State College, Kansas State College, Univ. of Maryland, Univ. of Massachusetts, Michigan State Univ., Univ. of Minnesota, Mississippi State Univ., Univ. of Missouri, Univ. of Nebraska, North Carolina State College, Ohio State Univ., Oklahoma State Univ., Pennsylvania State Univ., Purdue Univ., South Dakota State College, Univ. of Tennessee, A & M College of Texas, Texas Technological College, Virginia Polytechnic Institute, Washington State College, West Virginia Univ., Univ. of Wisconsin, and the Univ. of Wyoming.

The following is the list of those who won high standings in the contest:

All Products

Individuals

1. Donald J. Benning, University of Minnesota
2. Philip R. Lucas, University of Minnesota
3. William H. Schulz, University of Minnesota
4. Henry J. Sausen, Jr., University of Wisconsin
5. Wilfred B. Young, Jr., University of Connecticut
6. Lehmann D. Hines, Oklahoma State University
7. Sy Levine, Cornell University
8. Howard Eastham, California State Polytechnic College
9. Max A. Gonzenbach, Michigan State University
10. David A. Siegmund, Michigan State University

Teams

1. University of Minnesota
2. Michigan State University
3. University of Connecticut
4. Cornell University
5. Mississippi State University
6. California State Polytechnic College
7. South Dakota State College
8. Pennsylvania State University
9. University of Illinois
10. Oklahoma State University

Ice Cream

Individuals

1. Peter F. Pierpont, Connecticut
2. Keith P. Burnquist, California
3. Howard A. Eastham, California
4. John M. McGill, Wyoming
5. Donald J. Benning, Minnesota
6. Wilfred B. Young, Jr., Connecticut
7. Sy Levine, Cornell
8. John P. Garber, Virginia
9. Henry J. Sausen, Jr., Wisconsin
10. Joe E. Schuch, South Dakota

Teams

1. California
2. Connecticut
3. Wyoming
4. Minnesota
5. South Dakota
6. Clemson
7. Pennsylvania
8. Mississippi
9. Maryland
10. Tennessee

Milk

Individuals

1. Max A. Gozenbach, Michigan
2. William H. Schulz, Minnesota
3. Philip R. Lucas, Minnesota
4. Clifford G. Gerbig, Purdue
5. Thomas Ravencroft, Purdue
6. Howard A. Eastham, California
7. Robert L. Gromko, Washington
8. Dale L. Wolfe, Purdue
9. James B. Purcell, Nebraska
10. Donald J. Benning, Minnesota

Teams

1. Minnesota
2. Purdue
3. Georgia
4. Michigan
5. Texas
6. Pennsylvania
7. North Carolina
8. Cornell
9. Connecticut
10. Nebraska

Cheese

Individuals

1. Robert L. Gromko, Washington
2. William H. Schulz, Minnesota
3. Henry J. Sausen, Jr., Wisconsin
4. Sy Levine, Cornell
5. Donald J. Benning, Minnesota
6. Ralph D. Peterson, Cornell
7. Roland S. Golden, Mississippi
8. Albert Linden, Illinois
9. David G. Stecher, Washington
10. Joe E. Schuch, South Dakota

Teams

1. Minnesota
2. Washington
3. Cornell
4. Mississippi
5. Michigan
6. South Dakota
7. Pennsylvania
8. Illinois
9. North Carolina
10. Kansas

Butter

Individuals

1. Ross R. Wagner, South Dakota
2. Phil R. Lucas, Minnesota
3. Ralph D. Peterson, Cornell
4. Lehmann D. Hines, Oklahoma
5. Roland S. Golden, Mississippi
6. Richard Scanlan, Cornell
7. Donald J. Benning, Minnesota
8. Robert D. Rauch, Iowa
9. Wilfred B. Young, Jr., Connecticut
10. Max A. Gonzenbach, Michigan

Teams

1. Minnesota
2. Cornell
3. South Dakota
4. Michigan
5. Connecticut
6. Oklahoma
7. Iowa
8. Illinois
9. Missouri
10. Massachusetts

Ralston Fellowships Announced

Ralston Purina Company research fellowship awards have been announced for the school year, July 1, 1959, through June 30, 1960.

The objective of these awards is to assist in the training of additional personnel for leadership in the science of livestock and poultry production. The awards are for $1,800 annually.

Awards will be made in the fields of (1) nutrition and physiology research as applied to dairy, poultry, and animal husbandry, and (2) research in transmissible diseases of livestock and poultry.

Any individual qualified for graduate study in any Land Grant agricultural college or approved veterinary college (Canadian colleges included) who possesses desirable personal qualifications and submits a completed application may be eligible. The application and required information must be in the hands of the Awards Committee by March 1, 1959.

The selection of the recipients of these awards, as well as the rules governing the awards, shall be made by a committee of an

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officially appointed representative of the following organizations: Poultry Science Association, American Veterinary Medical Association, American Dairy Science Association, American Society of Animal Production, American Association of Land Grant Colleges and State Universities, and the Ralston Purina Company.

The committee consists of C. A. BRANDLY, Univ. of Illinois, American Veterinary Medical Association; ROBERT MACVICAR, Oklahoma State Univ., American Association of Land Grant Colleges and State Universities; L. E. HANSON, Univ. of Minnesota, American Society of Animal Production; J. B. FRYE, Louisiana State Univ., A.D.S.A., and R. H. THAYER, Oklahoma State Univ., American Poultry Science Association.

Application blanks for these fellowship awards may be obtained by writing the Ralston Purina Research Awards Committee, in care of J. D. SYKES, Ralston Purina Company, St. Louis 2, Mo.

W. C. Brown Named Secretary of National Dairy Research Center

W. CARSON BROWN has been named secretary of the National Dairy Research Center, Glenview, Ill.

Dr. Brown joined the company in 1948 as a project leader and has recently been working as assistant to the director. He is a graduate of West Virginia Univ., where he majored in dairy manufacturing. He received his Ph.D. from there in 1937.

Dr. Brown is a member of A.D.S.A. and the Institute of Food Technologists.

Dairy Technology Society News

Atlanta—WILLIAM KIZER, division manager of Coble Dairy Cooperative, Inc., Lexington, N. C., spoke at the January meeting of the group on the topic, "Conditioning for Operating Control."

Minnesota—"The Significance of Recent Research on Milk Proteins to the Dairy Industry," was the topic of ROBERT JENNESS, Univ. of Minnesota, at the January meeting of the society. At the same meeting, E. L. THOMAS, Univ. of Minnesota dairy judging coach, was also recognized for his fine work with the Minnesota team which won the 24th Collegiate Student's International Contest in Judging Dairy Products.

Metropolitan—The Metropolitan Dairy Technology Society—serving New York City and the surrounding metropolitan area—heard C. O. BALL, Food Science Dept., Rutgers Univ., New Brunswick, N. J. His topic was, "Principles Involved in Establishing Standards for Pasteurizing Milk."

Kansas—J. E. EDMONSON, Univ. of Missouri Dairy Dept., spoke on "Factors Affecting Fat and Solids Content of Milk." New officers presiding at the January meeting were GUY DUNCAN, president; JEFF STOVALL, first vice president; AL EDMUNDSON, second vice president; GUS SAUER, sergeant at arms, and T. J. CLAYDON, secretary-treasurer.

Detroit—The Detroit Society held a joint meeting in January with the Ice Cream Manufacturers of Metropolitan Detroit. Both groups installed new officers and held brief business meetings.

Michiana—ERLE KIGHTLINGER, Indianapolis attorney for the Unfair Milk Practices Act, spoke at the January meeting on the topic, "Outlook for Dairy Industry—Unfair Practices Act."

Central Michigan—Selling and salesmanship were two topics discussed by the main speaker at the January meeting of the Society. The speaker was Dr. A. CONRAD POSZ, Communications Skills Dept., Michigan State Univ., East Lansing. New officers for 1959 also were installed.

Western Michigan—CARL HALL, Michigan State Univ. Dept. of Agricultural Engineering, spoke at the January Society meeting on the topic, "Dairy Plant Equipment and Engineering in the U.S.A. and U.S.S.R." Officers nominated for 1959 are DON POPE, vice-president; DON MURRAY, secretary; WILLIAM KRUEGER, treasurer; RAY HUGHES, sergeant at arms; JACK JOPPE, director, and GLEN KLOPFENSTEIN, director.

Tri-Cities—Louisville, Ky. was the site of the January meeting of the Society. W. K. MOSLEY of the Mosley Laboratory spoke on the topic, "Plant Shakedown."

North Carolina—"The Latest Developments in Manufacturing Cottage Cheese," was the topic at the January meeting of the Society. WILLIAM B. THOMPSON, Consolidated Badger, Inc., West Depere, Wis., was the speaker. Newly elected officers for 1959 are MAX RUSSELL, president; HENDERSON SCOTT, vice president; VERNON WEAVER, treasurer, and W. M. ROBERTS, secretary.

Recent Death

EDMUND W. CHENG, 42, assistant professor of dairy science at Iowa State College, Ames, died Oct. 6, 1958.

A graduate of Southwest Associated Univ. of China, Dr. Cheng received his M.S. degree from Tsing Hua Univ. of China and his Ph.D. degree from Iowa State. He has been on the staff at Iowa State since 1951. He was the author of 30 scientific papers mostly in the fields of rumen physiology and the nutritional significance of estrogens in cattle.

He is survived by his wife, DOROTHY W. CHENG, Iowa City, Ia.

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Dairy Engineering Meeting Held Feb. 26-27 at Michigan State

The Seventh Annual National Dairy Engineering Conference was held Feb. 26-27 at Michigan State Univ., East Lansing. Cooperating on the program were the Depts. of Agricultural Engineering and Dairy.

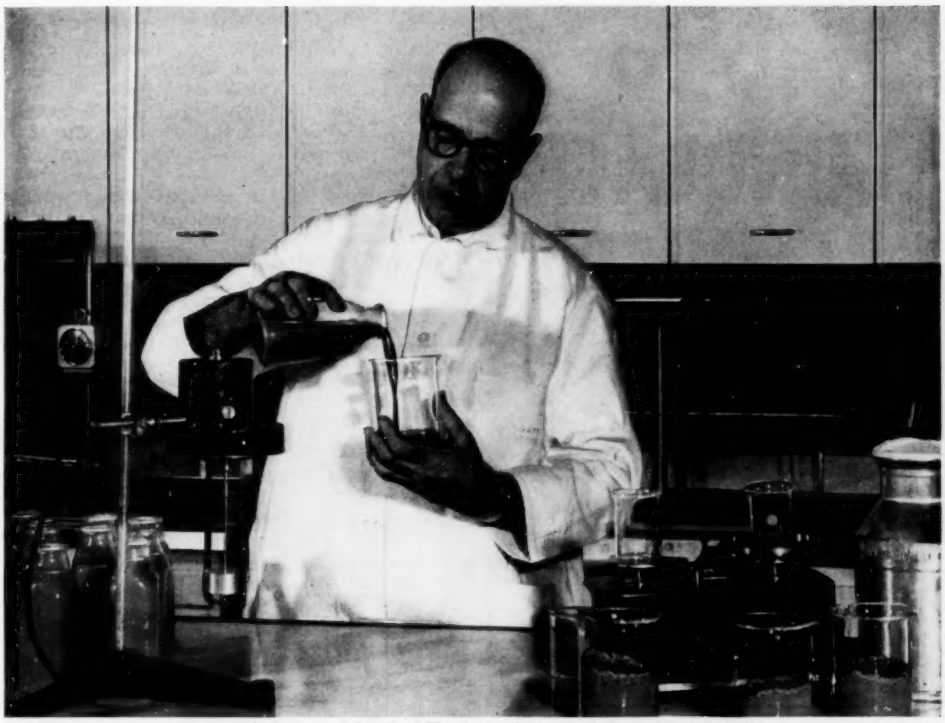
Theme of the conference was "More Profits Through Better Engineering." Sessions were devoted to papers and panels on "Over-all Plant Applications," "Processing Equipment," "Utilities," and other subjects. The program was as follows:

Over-all Plant Applications—A. W. FARRALL, Head, Dept. of Agricultural Engineering, Michigan State Univ., chairman; "How Management Can Reduce Costs," ROGER SHARKEY, Purepak Division, Excello Corp., Detroit; "Ice Cream Plant Labor Efficiency Studies," R. A. KEPPELER, Agricultural Engineering Dept., M.S.U.; "New Conveyor Developments," V. C. HECKMAN, Conveyor Specialties Co., Chicago.

Processing Equipment—T. I. HEDRICK, Dairy Dept., M.S.U., chairman; "Systems and Equipment for Dispenser Cans," E. R. EDMUNDS, R. G. Bright Co., Buffalo, N. Y., and GLENN JOHNSON, Ft. Wayne, Ind., Dairy Equipment Co.; "Making Canned Whole Milk," H. C. JACKSON, Dept. of Food Technology, Univ. of Wisconsin, Madison; "New Ideas in Evaporators and Dryers," J. P. TERRETT, Rogers Dryer, Detroit; "Operation of Triple Barrel Freezers," G. O. WYMOND, Cherry-Burrell Corp., Cedar Rapids, Ia.; "New Developments in Equipment for Casing of Bottles and Cartons," GAIL RIPMA, Cherry-Burrell Corp., Cedar Rapids, Ia.

Utilities—A. L. RIPPEN, Agricultural Engineering Dept., M.S.U., chairman; "Will Liquid Ammonia Positive Circulation Refrigeration Systems Improve Your Plant?", BILL RICHARDS, H. A. PHILLIPS C., Chicago; "Recent Developments in Automatic Cleaning of Storage Tanks," VINCENT MYERS, Girton Mfg. Co., Millville, Pa.; "Field Experiences with Air Operated Valves for CIP," DALE SEIBERLING, Klenzade Products, Inc., Beloit, Wis.; "What We'd Do Differently," a panel discussion consisting of TOM MCLAUGHLIN, Sealtest, Detroit, VALKER WEBB, McDonald Dairy Co., Flint, Mich., ROBERT GROSSNER, Deans Dairy, Rockford, Ill., and A. T. DALTON, Bowman Dairy, Chicago.

Friday Afternoon Session—C. W. HALL, Agricultural Engineering Dept., M.S.U.; "Ultra-High Temperature Processes," H. L. MITTEN, Creamery Package Mfg. Co., Chicago; "What's New in Packaging Cottage Cheese and Ice Cream," W. E. GUNNERSON, Anderson Bros. Mfg. Co., Rockford, Ill.; "Overhead Conveyors in Hardening Rooms," G. A. M. ANDERSON, The King Co., Owatonna, Minn.



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1. We start with top-grade ingredients. Kraft buys only the finest grade cocoa and quality sucrose. Because these ingredients must meet our rigid specifications, you can count on uniformity of color and flavor in the finished product.

2. We make our own stabilizer. Kraft extracts the stabilizer we use from Irish Moss which we harvest ourselves. Our special process assures thorough suspension with the viscosity you desire. You order the formula designed for your system and its high safety range allows adjustments in sweetness and strength without upsetting balance.

3. We manufacture in small batches. Unlike other manufacturers, Kraft makes chocolate powder only in small batches. This permits more exact control over quality. A sample from each batch of Kraft's powder is made into chocolate milk and its performance tested before any of the batch is shipped to dairies.

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F. R. ELLIOTT, Borden Company vice president and president of Borden's Milk and Ice Cream Company, has been elected a director of the company. He succeeds C. A. ECKBURG, who has resigned as a director. Mr. Eckburg has resigned also as Borden vice president for trade relations. However, he will continue with the company, as chairman of the directing board of the Wheeler Cheese Company, a Borden division.

Mr. Elliott, prior to assuming his present duties in 1957, was general manager of Borden's fluid milk operations, a post to which he was named in 1955. A law graduate of the College of William and Mary, he joined Borden's legal staff in 1929 after general law practice. He entered the operating phase of the company's business in 1932 as an officer of Borden's Farm Products Division in New York. Subsequently, he served in various executive positions with that division including its presidency.

Mr. Eckburg joined the Borden Company in 1928 in Wisconsin, his native state, after a number of years there in the cheese business. He was elected a vice president in 1943 and became a director of the company in 1947.

Illinois News

H. D. GRAHAM, a recent Ph.D. degree graduate of the Univ. of Illinois in dairy technology, has accepted a position with the Carver Foundation, Tuskegee Institute, Tuskegee, Ala. Dr. Graham will do research in food technology.

News from Beatrice Foods

THOMAS WHARTON has been promoted to manager of the Beatrice Foods Company plant at Zanesville, O. Mr. Wharton has been with Beatrice at Zanesville since 1942. He started in the Zanesville plant as a route driver-salesman, became route supervisor in 1946, and was named sales manager in 1949.

Florida News

H. H. WILKOWSKE, assistant director and dairy technologist of the Florida Agricultural Experiment Stations, has recently returned from a three-month assignment in Costa Rica.

From October through December, 1958, Dr. Wilkowske served as a cheese specialist to advise and assist the Costa Rican Ministry of Agriculture and Industries in the development of a cheese industry.

The assignment was made under the Univ. of Florida contract with the International Cooperation Administration.

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Department of Dairy Manufacturing
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Dear XXXXXX:

In answer to your letter of the 30th, we, at Creamery Package, offer several approaches to UHT through our CP Crescent and Crescent SC plate heaters with pressurized water heating units; our CP Swept Surface heater; and our CP Steam Infuser (direct contact steam heater). In addition, CP Vac-Heat units are offered for UHT or as accessory flavor treating units to UHT pasteurizers.

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Although CP can furnish equipment to heat to any temperature up to 300°F. and above, we suggest that fluid milk processors start near the lower temperature limits of the UHT range, for this will assure them of an extension of shelf life consistent with present methods of post-pasteurization handling and will yield excellent flavor. CP installations can be modified for higher temperatures without loss in original investment at a later time if basic research proves that higher temperatures are really beneficial.

Your comments and questions were most welcome.

Sincerely yours,

H. L. Mitten, Jr.

H. L. Mitten, Jr.
Assistant to Vice President

HLM:jkw

STUDENT CHAPTER NEWS

A Section Devoted to News of Student Activities

J. E. JOHNSTON, *Editor*

V.P.I. Dairy Club News

The Virginia Polytechnic Institute Student Chapter of the American Dairy Science Association was honored this year when four of its members were nominated to "Who's Who in American Colleges and Universities." A total of thirty-four outstanding students were selected from V.P.I.

Those receiving this high honor from the chapter were: Miss Judith M. Hess, Robert E. Benton, John P. Garber, and William M. Harris, Jr. Not only have these students excelled in scholarship but they have shown outstanding accomplishments and leadership abilities.

Miss Judith M. Hess is a dairy production student from Titusville, New Jersey. She has been a member of the dairy club for four years, serving as secretary her junior year and editor of the club annual this year. She is a member of the Phi Sigma Society and is serving as secretary of Pi Delta Epsilon this year. She has served as associate editor of "The Tech-nique" for three years and was on the "Virginia Tech" staff one year. She is a member of the Westminster Fellowship, 4-H Alumni Club, and Hillcrest Dorm Club. She participated in intramural basketball and was a member of the 1957 dairy products judging team. Her achievements are highlighted by being the first co-ed to receive the Borden Scholastic Award at V.P.I.

Robert E. Benton is a dairy production student from Union Hall, Virginia. He is president of the following: Class of '59, Dorm Club, the Civilian Student Body and Chancellor of Alpha Zeta. He is a member of Omicron Delta Kappa, Dairy Club, Cotillion Club, Student activities committee, Board of Directors of the V.P.I. Athletic Association, V.P.I. Precentennial Development Committee, Franklin County Club, Biology Club, and Inter-Dorm Club Council. He has served on the Agriculture Exposition display committee, Class of '59 ring committee, radio program committee organization of dairy club annual committee, and has been a delegate to Alpha Zeta National Conclave, National Students Association Regional Conference, Seminar for Virginia Colleges, and United Nations Seminar.

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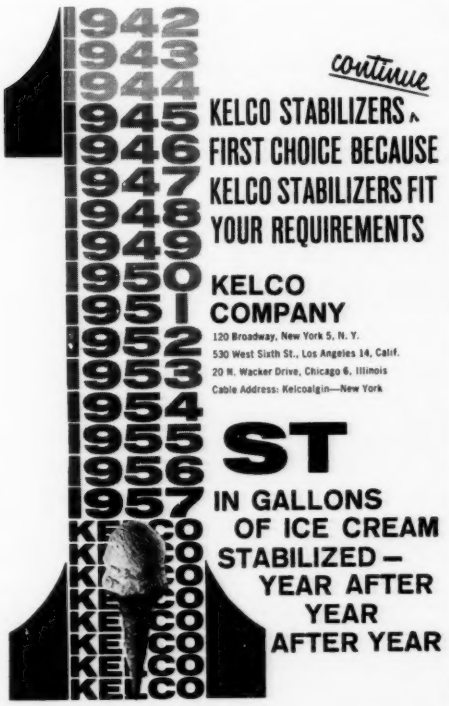
John R. Garber, a production student from King William, Virginia, has been an active dairy club member for four years and has served as treasurer and vice-president. He is a member of the 4-H Alumni Club, Cafi Club, Westminster Fellowship, Alpha Zeta, Block and Bridle, and Phi Sigma Society. He was a member of the dairy cattle judging team in 1957 and was fifth high individual in judging of all breeds at Eastern State Exposition at Springfield, Massachusetts. He has shown an animal in the dairy show for the last three years and has worked part time at the dairy barns since entering school. He is the recipient of the Smith-Douglas 4-H Scholarship and the Ralston-Purina Scholarship.

William M. Harris, Jr. is a production student from Spottswood, Virginia. He is a four year member of the dairy club and is serving as secretary this year. He is also a member of the 4-H Alumni Club for which he has served as secretary and president. He is a member of Block and Bridle, Cafi Club, and Westminister Fellowship. He has served on the publicity and picnic committees for the Agriculture Exposition, shown an animal in the dairy show for three years and was a member of the 1958 dairy cattle judging team. He is the recipient of the Sears Roebuck



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L.S.U. Dairy Science Club News

The L.S.U. Student Affiliate Chapter activities on campus got off to a slow start this year due to the fact that the L.S.U. Dairy Cattle Judging team included three club officers: Gerald Phares, President; Jerry Robinson Jr., Secretary; and Harry Landry, Vice President. The team is sponsored by the club which assists by paying a portion of the teams' expenses to judging contests. The team, coached by George Hindery, Instructor in Dairying, participated in three contests this fall. It was 1st place team over-all, of 10 participating teams, at the Southern Intercollegiate Contest held at Memphis, Tennessee. It was also 1st place team in judging Guernseys and Brown Swiss with individual honors going to: Phares 1st in Brown Swiss and 7th in the contest; Landry 3rd in Guernseys and 5th in the contest; and Robinson 3rd in Brown Swiss, 4th in Holstein, 4th in Jersey and 2nd in the contest. The team then went on to the National Intercollegiate contest at Waterloo, Iowa where it placed 15th over-all of 31 teams represented. Individual honors were won by Jerry Robinson, Jr. who was 1st place individual in Jerseys and tied for 4th in giving oral reasons on all breeds.

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Not content with this, the team went on to the International Dairy Cattle Exposition in Chicago where it placed 11th of 15 teams. Individual honors were again won by a team member, however, when Gerald Phares was 1st individual in judging Jerseys and 9th over-all.

With judging contests over, the on campus activities of the club got under way. Club members participated in the activities associated with the National American Dairy Association "Dairy Princess Contest" held in Baton Rouge November 11 through the 14th. The club was honored by having one of its members, Miss Judy Cook of Ringold, La., participating as the Louisiana "Dairy Princess." While Judy didn't win the contest she gave a fine account of herself. Other fall activities included an open house program for new Freshmen, fall initiation and a Christmas Party and dance. The Christmas party held the evening of December 11th was a huge success with Santa distributing gifts to Faculty, their families, and students.

News from the O.S.U. Dairy Tech Club

The Ohio State Dairy Tech Club co-operated with the Department of Dairy Technology in the arrangement and management of the 10th annual Dairy Tech Homecoming Celebration, October 25, 1958. The members of the club served on various committees such as registration, hospitality, and food serving committees. Ronald Walters, senior in Dairy Tech and immediate past President of the Club presided at the brunch. Paul Culler, President of the Club, spoke briefly on the affairs of the Club during the past year and introduced the 1958 Dairy Products Judging Team. Former students and club members who represented their classes at the celebration were Keith A. Baylor, Class of 1948; I. Tom Swartwood, 1938; Virgil Dreyer, 1928.

Some of the O.S.U. Dairy Tech students that attended the Homecoming Celebration. Shown in the picture are (left to right): Ed Geishecker, Graduate Research Assistant; Jude Aubry, Fresh.; Keith Jenkins, Fresh.; Rodger Hagmaier, Fresh.; and Paul Rogers, Fresh.

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The feature speaker at the November meeting of the Ohio Dairy Tech Club meeting was Mr. Gene Lower, a 1950 Dairy Tech graduate of Ohio State, who spoke on the opportunities for graduates in the industrial phase of Dairy Technology and stressed the importance of a well-rounded education so that students would be better suited to meet the industry's requirements. He also related some of his experiences since graduation and told about some of the problems encountered in day-to-day plant operations. A discussion period and refreshments followed the presentation.

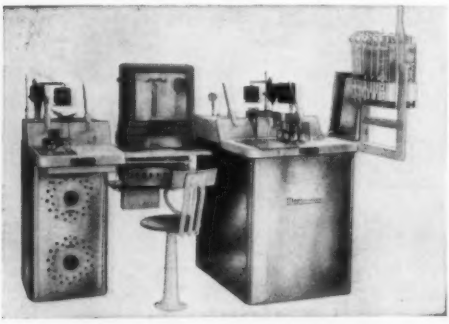
National Dairy Products Corporation Promotes Purko, Walker, White

The National Dairy Products Corporation has announced the promotion of three of its scientific people, MYROSLOW PURKO, R. G. WALKER, and J. F. WHITE.

Dr. Purko, who received his Ph.D. from the Univ. of Illinois, has been promoted from bacteriologist to group leader. He joined the company in 1954.

Mr. Walker has been promoted from senior food technologist to group leader in the Fruits and Vegetables Laboratory. He is a graduate of the Univ. of Wisconsin.

Mr. White has been promoted from junior food technologist to group leader in the Fruits and Vegetables Laboratory. He graduated in dairy industry from the Univ. of Wisconsin.



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SOME PHYSICAL PROPERTIES OF MILK. V. EFFECTS OF AGE ON THE VISCOSITY OF PASTEURIZED FRACTIONS OF MILK [1]

C. H. WHITNAH, W. D. RUTZ,[2] AND T. G. ALEXANDER[3]
Department of Chemistry and of Dairy Husbandry
Kansas State College, Manhattan, and
Eastern Utilization Research Branch, USDA, Washington, D. C.

SUMMARY

The relationship between changes in viscosity and age after pasteurizing has been studied for (1) separated milk, (2) "wheys" obtained by centrifuging out as sediments all or part of the caseinate-complex, and (3) fractions of this sediment resuspended in water.

Aliquots of wheys and of resuspended sediments were pasteurized at 61.7° C. for 30 min. Viscosities were measured at 4° C. after storage for various times at 2–4° C. For pasteurized wheys the increase of viscosity with age was less, as a larger fraction of the caseinate sediment had been removed. The resuspended sediment fractions all increased in viscosity with age. The major part of each increase was recovery from decreases in viscosity on pasteurization.

The object of this study was to determine which fractions of pasteurized milk are responsible for the increase of viscosity with age. Age has been defined (5) as the interval from the time the pasteurized fraction was cooled until the time viscosity was measured. Effects of age on the viscosity of pasteurized whole milk have been discussed (5).

EXPERIMENTAL PROCEDURE

The general procedure in this study was to obtain milk fractions by centrifuging, and to determine which fractions change in viscosity with age. The viscometers previously described (5) were used in the present study. The temperature control used in the present study also has been described (4). The milk fractions used were obtained from one or more of three centrifuges, including the separator.

The first fractions were prepared in Washington, D. C., with a Ford centrifuge (1). Fresh, whole milk was centrifuged a few seconds and a small amount of milk added to the spinning bowl to displace fat. This procedure was repeated until a pooled sample of several liters of skimmilk was obtained. An aliquot of this pool constituted 100% of the original nitrogen fraction. The other fractions were prepared by spinning other aliquots of the pool for various lengths of time up to 70 min. Samples of about 120 ml. of these fractions were packed in ice and shipped by air to the Kansas State College laboratory.

Received for publication July 7, 1958.

[1] Contributons No. 573 and No. 266 from the Kansas Agricultural Experiment Station, Manhattan.

[2] Present address: Fairmont Foods, Omaha, Nebraska.

[3] Present address: Food and Drug Adminstration, Washington, D. C.

Larger liquid fractions, and fractions of reconstituted sediments, were prepared at Kansas State College. The skimmed milk for these fractions was prepared by separating four times fresh milk, still naturally warm, in a small commercial cream separator. Fractions of the caseinate complex and of the centrifuge whey were obtained at Kansas State College with a Sharples Supercentrifuge, by procedures similar to those used by Hauser and coworkers (2) for fractionating clay suspensions. Similar fractionations of milk also have been studied at the Dairy Products Laboratory (3). A peristaltic pump was used to feed the Sharples centrifuge at well-controlled rates of 10–20 ml/min. This pump, made by the American Instrument Co. of Silver Spring, Md., was modified by replacing the synchronous motor with a larger one, where the speed was controlled with a transformer. The rollers acting on the tube were then tightened, so that flow rate was nearly independent of supply level. The centrifuge bowl was also surrounded by a refrigerated jacket, so that material with an input temperature of 4° C. could be recovered at about 6° C. It was not possible to obtain as complete removal of casein as with the Ford centrifuge, but it was possible to obtain within 2–7 hr. fractions which were large enough to permit the study of viscosity up to the ages of 300 hr. The sediment fractions were scraped first from the lower portion of the bowl, then from progressively higher portions. Each fraction was reconstituted with water and analyzed for nitrogen.

Aliquots of wheys and of resuspended sediments were pasteurized at 61.7° C.

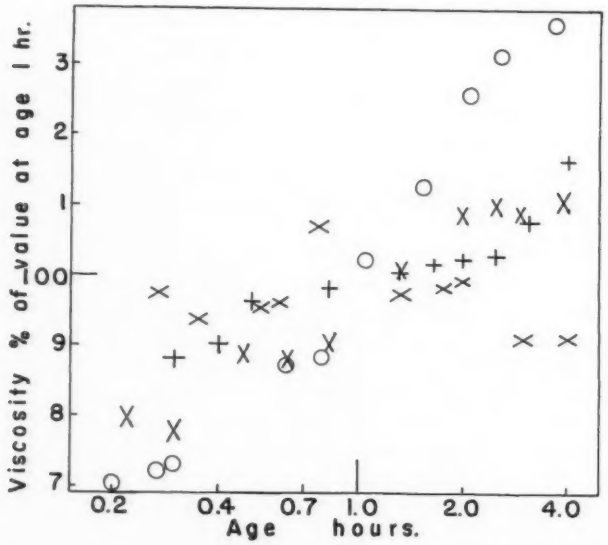

FIG. 1. Viscosity as percentage of value at age 1 hr. vs. age 0.1–5.0 hr., after pasteurization for four liquid fractions of skimmilk prepared in Ford bowl centrifuge: ○ = 100% of original nitrogen; X = 71.4%; + = 55.0%; × = 32.3%.

for 30 min. Viscosities were mesaured as previously described (5) at 4° C. after storage for various lengths of time at 2–4° C.

RESULTS

The viscosities as percentage of the values at age 1 hr. are shown (Figures 1 to 6). In these figures, the viscosities as ordinates are plotted aganist the logarithm of the age as the abscissas.

For pasteurized wheys (Figures 1 and 2), the increase of viscosity with age was less, as a larger fraction of the sediment had been removed.

For reconstituted sediments, the results were less consistent. It was still true (Figures 3, 4, 5, and 6) that the increase of viscosity with age depended largely on the concentration of nitrogen in the suspension. (Note the sensitive viscosity scale in Fgure 4.) If the change of viscosity with age is to be less for small particles than for large ones, then the viscosity at any age (as per cent of value at 1 hr.) for particles of intermediate size should be intermediate be-

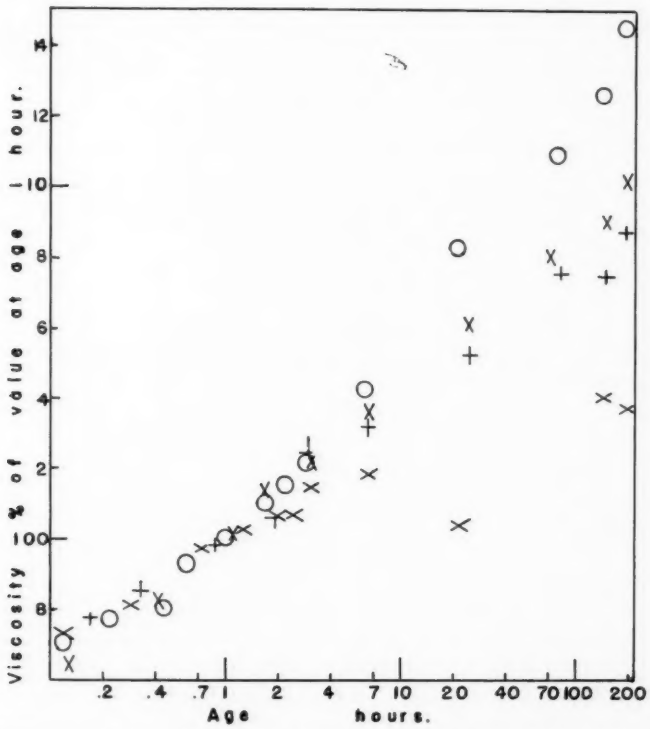

FIG. 2. Viscosity as percentage of value at age 1 hr. vs. age 0.1–200 hr., after pasteurization for four liquid fractions of skimmilk prepared in Sharples supercentrifuge: ○ = 100% of nitrogen in original skimmilk; × = 84.4%; + = 61.0%; × = 42.4%.

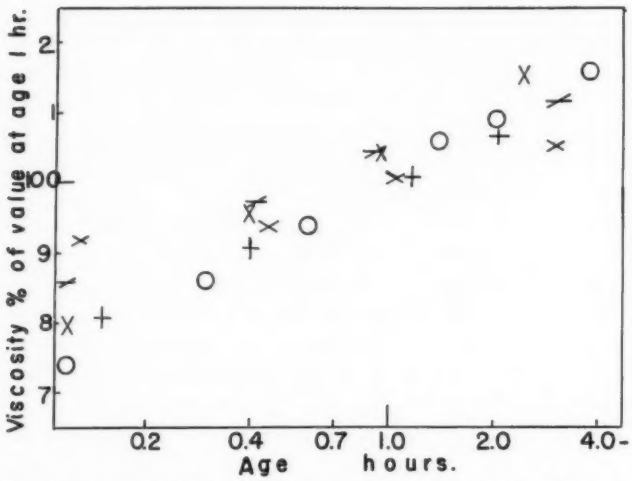

FIG. 3. Viscosity as percentage of value at age 1 hr. vs. age 0.1–4.0 hr., after pasteurization for five reconstituted fractions of sediments of skimmilk from progressively higher regions of a Sharples supercentrifuge bowl, after feeding milk at 10/ml/min through the bowl, running at 830 r.p.s.: ○ = 87.4% of nitrogen in the skimmilk; X = 83.2%; + = 70.6%; × = 63.9%; × = 78.3%.

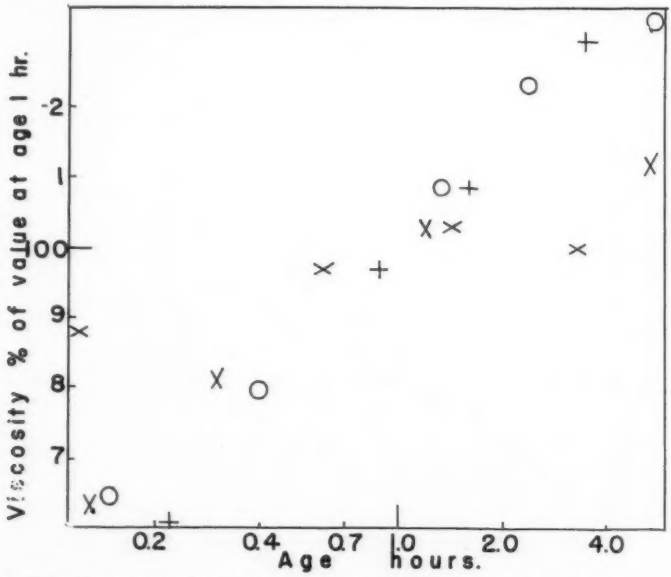

FIG. 4. Viscosity as percentage of value at age 1 hr. vs. age 0.1–5.0 hr., after pasteurization for four reconstituted fractions of sediment of skimmilk from progressively higher regions of a Sharpless supercentrifuge bowl, after feeding milk at 20/ml/min through the bowl, running at 430 r.p.s.: ○ = 21.0% of nitrogen in the skimmilk; X = 19.8%; + = 20.0%; × = 20.5%.

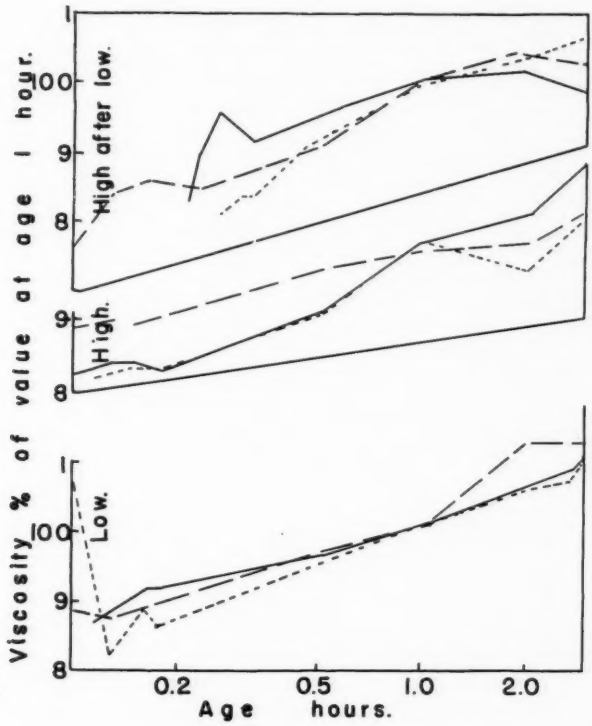

FIG. 5. Viscosity as percentage of values at age 1 hr. vs. age 0.1–3.0 hr., after pasteurization for reconstituted sediments of skimmilk. The centrifuge speeds were, respectively, low, high, and high after low. The sediment after each run was divided into three parts, the largest particles—small dashes, the intermediate particles—full line, and the smallest particles —long dashes.

tween the values for small particles and for large particles. In some instances (Figure 4), it was found that the change of viscosity with age was less for small particles than for large particles. For other samples (Figures 5 and 6), the intermediate viscosities were for particles of intermediate size at only certain ages. At low speed in the age range 3–40 hr., the viscosity increased progressively with particle size. In the age range 0.5–3 hr., the largest particles had the smallest viscosity and the smallest particles the largest viscosity. At the other two speeds, relations to particles size were less consistent.

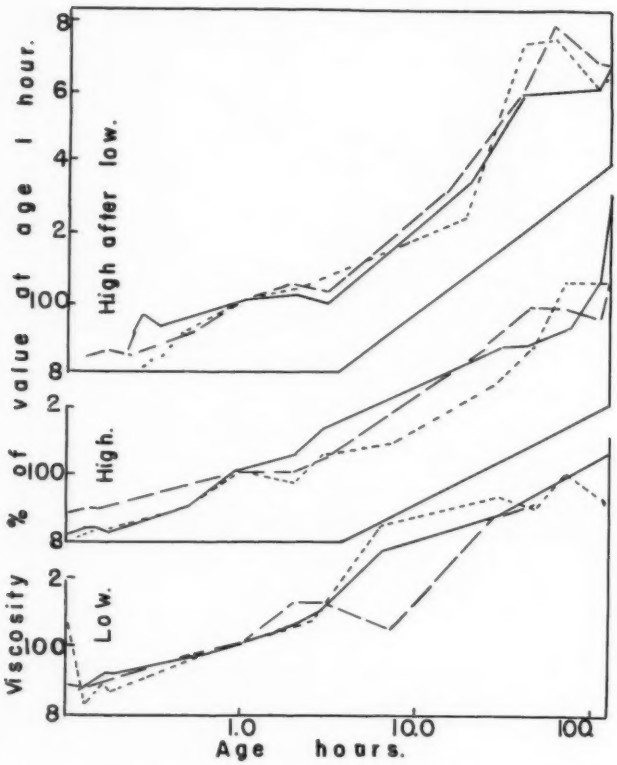

FIG. 6. Viscosity as percentage of value at age 1 hr. vs. age 0.1–100.0 hr., after pasteurization for reconstituted sediments of skimmilk. The centrifuge speeds, were, respectively, low, high, and high after low. The sediment after each run was divided into three parts, the largest particles—small dashes, the intermediate particles—full line, and the smallest particles—long dashes.

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SOME PHYSICAL PROPERTIES OF MILK. VI. THE VOLUMINOSITY OF CASEINATE COMPLEX IN MILK, AND RECONSTITUTED SEDIMENTS [1]

C. H. WHITNAH AND W. D. RUTZ [2]

Departments of Chemistry and of Dairy Husbandry, Kansas State College, Manhattan

SUMMARY

Six liquids ranging from 1.80 to 0.0% casein were prepared by centrifuging a sample of milk. One fraction of sediment was reconstituted with water to contain 5.325% casein. Half of this suspension was diluted with an equal weight of water, and such dilutions repeated until six concentrations of sediment were obtained. Viscosities were measured at 15 temperatures from 4 to 70° C. At each temperature in each of four series of five or six pairs of values for relative fluidity and casein concentration, the slope $d(\phi/\phi')/dC$ was calculated by least squares. Voluminosities were then calculated by the equation $V = 0.4\ d(\phi/\phi')/dC$. Approximate average values of V ranged from 6.45 ml/g at 4° C. to 2.24 ml/g at 70° C., when ϕ' was the fraction containing least casein. Corresponding values when ϕ' was water were 5.42 and 2.95 ml/g.

Voluminosity has been defined as the volume which 1 g. of the substance occupies in its disperse form (3). There is some uncertainty as to the meaning of the word "occupy." Eucken (5) used the expression "partial ionic volume" to denote volume occupied. He listed the following four terms contributing to partial ionic volume: *(a)* the volume occupied by the unhydrated ion; *(b)* volume change due to structural changes in solvent (solvent effect); *(c)* "electrostriction," or decrease in volume of the solvent molecules attached to the ion; and *(d)* a volume increase due to the formation of a second diffuse hydration layer around the ions. Buchanan *et al.* (2) discussed ways in which water is bound to and distributed in or around protein particles. Ogston (9) stated that the particle of solute is likely to interact with the solvent in several ways: by viscous interaction, which leads to movement of solute; by chemical or physico-chemical interaction, such as hydrogen bonding; by electrostriction, leading to a more or less firm attachment of solvent to solute, and by physical entrainment, which is really the same as viscous interaction, but is regarded as a carriage of excess solvent due to irregularities of the shape of the solute particle. McMeekin *et al.* (8) compared or contrasted several methods for determining the water content of protein crystals. Wang (10) referred to three effects of rate of diffusion: "obstruction," "direct hydration," and "breaking down of water structure." He also found variations of instantaneous exchange of labeled water between bound and free water.

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[1] Contributions No. 572 and No. 265 fom the Kansas Agricultural Experiment Station, Manhattan.

[2] Present address: Fairmont Foods, Omaha, Nebraska.

Ford (6) has used voluminosity, Einstein's Equation (4), and later related studies to develop the equation $V = 0.4\ d(\phi/\phi')/dC$. In this equation, V = voluminosity, ϕ = fluidity of the suspension, and ϕ' = fluidity of the solvent, C = (% casein N × 6.89 × 0.697 × density of solution)/100.

The object of this study was to apply Ford's equation, over the range of temperatures where temperature effects on the casein in milk are minimum, to two series of casein concentrations. In one series, the suspensions were milk from which increasing amounts of casein-containing sediments had been centrifuged out. In the other series, the middle fraction of such sediment was resuspended in varying amounts of water.

PROCEDURE

A sample of fresh milk was collected at the outlet from a pipe-line milker. While the milk was still warm, it was put four times through a small commercial separator. This removed most of the fat and some of the larger caseinate particles, as well as any leucocytes or other large foreign protein particles. Part of Liquid 1 (the resulting skimmilk) was fed through a Sharples Supercentrifuge, with internal bowl dimensions of length, 20.32 cm., and diameters, 4.4350 cm. in the body and 2.1460 cm. at the overflow weir, at the rate of 15 ml/min while the centrifuge was running at 333 r.p.s. This separation produced Liquid 2. The sediment lining the bowl was not used in this study. Liquid Fraction 4 and Sediment Fractions 4–1, 4–2, and 4–3 were similarly obtained, using a feed rate of 10 ml/min and a separator speed of 833 r.p.s. The coarsest sediment fraction, 4–1, was scraped from the bottom third of the bowl first, Fraction 4–2 from the middle third second, and Fraction 4–3 from the top last. Sediment 4–2 was reconstituted with water to contain 5.325% casein. Half of this suspension was diluted with an equal weight of water. Such dilutions were repeated until six concentrations of sediment were obtained. Sediment 4–2 was the only one used for this study. Liquid 3 was obtained by mixing equal volumes of 2 and 4. Liquid 5 was obtained in a manner similar to Liquid 4, starting with Liquid 2 instead of Liquid 1. Liquid 6 was obtained by centrifuging part of Liquid 4 for an hour at 500 r.p.s. in a Ford air-driven centrifuge (7).

The method of measuring viscosity has been previously described (11, 12). Viscosities of Liquids 1 to 6 and of the six concentrations of Sediment 4–2 were measured at 15 temperatures, from 4 to 70° C., as listed in Table 1. Fluidities ϕ/ϕ' were calculated relative to the fraction containing the least nitrogen, and to water. The slope of each series of five or six pairs of values for $\frac{d(\phi/\phi')}{dC}$ vs. nitrogen concentration was determined by least squares and converted to voluminosity using Ford's equation (6).

RESULTS AND DISCUSSION

Table 1 shows for the six liquids, the temperatures at which the viscosities were measured, the concentrations of casein, the viscosities, and the voluminosities V_L and V_W.

Table 2 shows similar data for the six sediments. Batchinski (1) found among about 80 organic liquids and molten salts that except for associated liquids, the plot of fluidity vs. specific volume was a straight line. The data in Tables 1 and 2 do not plot as straight lines. A smooth curvature could be ascribed to a gradual change in association.

The plots (Figure 1) approximate such a smooth curvature. Irregularities over short temperature ranges were much larger, however, than can be explained by the estimated errors of measurement (12). The word "association" as used here may refer either to polymerization of the casein particles or to changes in the amount of water bound as a hydrate or otherwise. The irregularities may indicate abrupt changes either in polymerization or in bound water at certain small intervals of temperature. These irregularities do not appear consistently on the four curves at any one temperature. Their relationship to other sediment fractions and to other samples of milk are subjects for future study.

TABLE 1

Viscosities and voluminosities of centrifuged liquid from skimmilk

Sample	1	2	3	4	5	6		
% casein	1.80	1.52	1.98	0.40	0.35	0.00		
t ° C.			Viscosity poises × 10^5				V_L	V_W
4	2,644	2,558	2,379	2,167	2,160	2,075	6.488	4.540
8	2,253	2,377	2.051	1,904	1,899	1,825	6.759	4.585
10	2,091	2,078	1,946	1,787	1,771	1,713	5.568	3.831
15	1,819	1,750	1,646	1,591	1,539	1,505	4.838	3.441
20	1,521	1,505	1,416	1,375	1,335	1,303	4.065	3.025
25	1,290	1,272	1,222	1,218	1,150	1,123	2.972	2.715
30	1,130	1,118	1,047	1,070	1,016	992	2.820	2.475
35	982	971	936	895	891	870	3.349	2.650
40	871	865	837	790	798	779	3.367	2.609
45	793	795	760	725	735	721	3.159	2.608
50	725	728	698	666	677	664	2.983	2.190
55	664	667	640	610	621	602	2.913	2.283
60	610	600	588	563	572	564	2.535	2.786
65	562	564	542	521	526	522	2.786	1.950
70	524	526	505	485	493	487	2.709	1.402

TABLE 2

Viscosities and voluminosities of reconstituted sediments from skimmilk

Sample	1	2	3	4	5	6		
% casein	5.325	2.663	1.3315	0.6657	0.3328	0.1664		
t ° C.			Viscosity poises × 10^5				V_L	V_W
4	4,206	2,352	1,858	1,679	1,592	1,585	6.140	6.240
8	3,513	2,046	1,529	1,487	1,410	1,400	5.939	6.011
10	3,217	1,865	1,534	1,387	1,326	1,320	5.808	5.887
15	2,595	1,578	1,332	1,227	1,171	1,155	5.435	5.521
20	2,102	1,357	1,156	1,065	1,029	1,014	5.027	5.111
25	1,705	1,151	944	937	907	889	4.599	4.754
30	1,435	989	874	828	803	791	4.340	4.493
35	1,183	805	764	724	710	698	3.885	
40	1,014	721	681	642	632	634	3.763	3.874
45	906	703	628	595	581	579	3.619	3.808
50	821	643	578	550	537	533	3.477	3.671
55	745	590	531	507	498	494	3.369	3.522
60	680	545	493	471	463	494	3.253	3.376
65	622	503	457	439	431	426	3.115	3.269
70	579	469	428	411	404	401	3.085	3.183

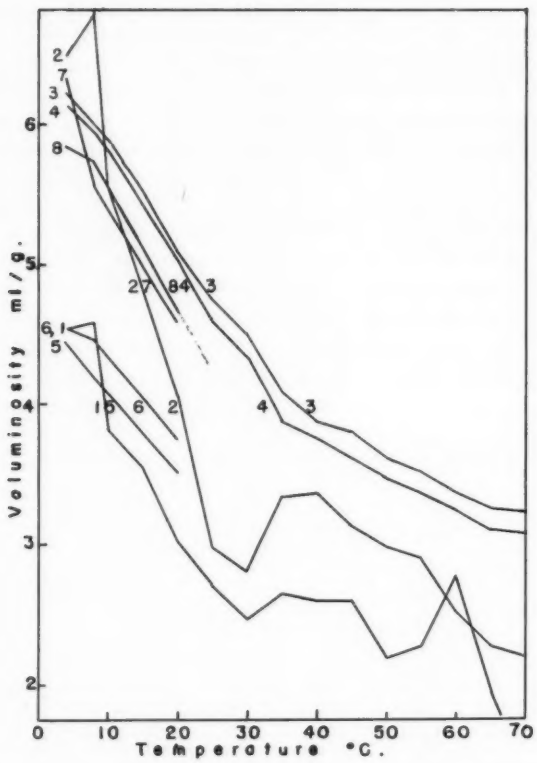

FIG. 1. Voluminosity vs. temperature for the caseinate complex in milk, and in centrifuge sediment unconstituted with water. Fresh: from Sharples supercentrifuge *(1)* liquid fractions, relative to water; *(2)* liquid fractions, relative to the fraction containing least nitrogen; *(3)* sediment relative to water; *(4)* same sediment as No. 3, but relative to most dilute fraction. From Ford bowl centrifuge: *(5)* liquids relative to water, 60 hr. after separation; *(6)* like No. 5, but 195 hr. after separation; *(7)* liquids relative to fraction containing least nitrogen, 60 hr. after separation; *(8)* like No. 7, but 195 hr. after separation.

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ESTIMATION OF THE MONOGLYCERIDE CONTENT OF MILK

R. G. JENSEN AND M. E. MORGAN

Department of Animal Industries, Storrs Agricultural Experiment Station, Storrs, Connecticut

SUMMARY

Existing methods for the estimation of 1-monoglycerides have been modified so that the formaldehyde produced by periodic acid oxidation is measured colorimetrically by reaction with chromotropic acid. Isomerization with perchloric acid was used to convert 2-monoglycerides to 1-monoglycerides. The formaldehyde was quantitatively recovered from commercial 1-monobutyrin, and from glycerol-free monoolein, 1-monolaurin, and 1-monopalmitin when chloroform solutions or purified butterfat containing 1.0 and 0.1% levels of monoglyceride were analyzed. Butterfat from both normal and rancid milk obtained by the silica-gel extraction method, employed in the determination of free fatty acids and cottonseed oil digested with pancreatic lipase, were analyzed for total monoglycerides. Normal milk contained from none to a trace when 10-ml. aliquots were analyzed. Acid degree increases ranging from 8.55 to 12.95 were accompanied but not paralleled by increases in 1-monoglyceride content ranging from 0.36 to 0.86 m*M* per 100 g. fat, and a 2-monoglyceride content ranging from 0.56 to 0.99 m*M* per 100 g. fat. The presence of diglycerides in rancid milk is indirectly indicated.

The estimation of 1-monoglycerides can be accomplished by the periodic acid method (14), in which the adjacent hydroxyl groups on the glycerol part of the molecule are oxidized, thus yielding water, 1 *M* of formaldehyde, and esters of hydroxyacetaldehyde. The remaining periodic acid is determined by an iodometric titration with thiosulfate. A modification of this method (10), which requires at least 3 mg. of 1-monoglyceride, was used by the authors in the detection of these compounds in the fat from rancid milk (9). However, the method has the limitation that periodic acid will react with substances containing adjacent hydroxyl groups, e.g., glycerol and carbohydrates. Coppock *et al.* (3) found, in addition, that many other substances including oleic acid and phospholipids interfere with the method and, if present, may yield erroneously high results. Furthermore, the method has been observed to yield blank values of up to 1.5% apparent 1-monoglyceride content on pure tri-, di, and 2-monoglycerides (12). Two-monoglycerides are not oxidized by periodic acid, but can be isomerized to 1-monoglycerides with perchloric acid, as described by Martin (10) and Brokaw *et al.* (2).

Coppock *et al.* (3) employed the periodic acid oxidation method of Handschumaker and Linternis (7), in which the monoglycerides are dissolved in chloroform and water added after the oxidation is complete. Formaldehyde arising from the oxidation of 1-monoglycerides was detected by treatment of the aqueous layer with chromotropic acid. Dowse and Saunders (4) recovered the formaldehyde formed in the oxidation by steam distillation and estimated it with chromotropic acid. Neish (13) employed chromotropic acid to determine the formaldehyde resulting from the oxidation of glycerol with periodic acid. In estimating the formaldehyde produced from 1-monoglycerides by periodic acid

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oxidation, the influence of some of the interfering substances mentioned by Coppock *et al.* (3) can be avoided. The only compounds which would cause interference are those which oxidize to form formaldehyde, such as glycerol and carbohydrates.

Since the partition coefficient of formaldehyde between water and chloroform is very low, 0.00785 for a 2.559% aqueous solution of formaldehyde (16), it follows that if water is added to the periodic acid-chloroform-monoglyceride reaction mixture, most of the formaldehyde should be extracted in the aqueous layer. The present paper describes a modification of the method of Martin (10) which will determine from 100 to 200 γ of 1-monoglyceride and its application to extracted milk fat.

EXPERIMENTAL PROCEDURE

Estimation of 1- and 2-monoglycerides (periodic–chromotropic acid method). One milliliter of a monoglyceride-chloroform solution which, upon oxidation, will yield 10–30 γ of formaldehyde is pipetted into a 22 by 175-mm. tube. Two milliliters of propionic acid [1] and 1 ml. of periodic acid solution are added, the tube swirled and allowed to stand for 15 min. One milliliter of sodium arsenite solution is added, the tube is swirled, and allowed to stand for 30 min. or until the iodine color has disappeared. Five milliliters of water is added, the tube is swirled vigorously, and the phases allowed to separate. A chloroform haze which persists in the aqueous layer will clear if the tube momentarily is placed in hot water. One milliliter of the aqueous phase is transferred to a 22- by 175-mm. cuvette and mixed with 10 ml. of chromotropic acid solution. The tube is placed in a boiling water bath for 30 min., cooled to room temperature, and the transmission measured in a colorimeter at 580 mμ. A reagent blank must be run with each set of determinations and a standard curve for each new batch of chromotropic acid.

The standard curve is prepared by weighing approximately 30 mg. of reagent-grade formaldehyde into a 100-ml. volumetric flask, adding 5 ml. of ethyl alcohol, and making up to volume with chloroform. Suitable dilutions are made with chloroform to obtain solutions containing from 10 to 60 γ per milliliter. The formaldehyde content of the reagent-grade solution is determined by the bisulfite method (16).

To determine total monoglyceride, add 0.003 ml. of 56% perchloric acid to each milliliter of chloroform-monoglyceride solution, as prepared above. Shake for 1 min. and allow to stand for 9 min. Add the periodic acid solution and proceed as described above. Calculate the total monoglyceride content by multiplying the result by 1.15. The 2-monoglyceride content can be obtained by subtracting the 1-monoglyceride content from the total monoglycerides.

Samples of crude 1-monobutyrin and glycerol-free 1-monolaurin, 1-monoolein, and 1-monopalmitin were analyzed by Martin's method (10) and by the procedure described above. Pure 2-monoglycerides were unavailable, so cottonseed oil digested with pancreatic lipase (11) was analyzed by the procedures above for 1- and 2-monoglycerides after isomerization, as described by Martin

[1] Propionic acid was substituted for acetic acid, because of the relatively large amounts of formaldehyde present in the latter (4).

(10) and Brokaw *et al.* (2). Recovery experiments were carried out on milk fat to which monoglycerides were added. Any monoglycerides originally present in the fat were removed, as described by Börgstrom (1). The fat treated by this method yielded no formaldehyde when tested by the procedure described above.

Materials:

(a) Chloroform is washed four times with distilled water, distilled over calcium chloride, and the required amount of ethanol added.

(b) Propionic acid—reagent grade.

(c) Periodic acid—167 mg. of periodic acid (H_5IO_6) is dissolved in 20 ml. of water and diluted to 100 ml. with propionic acid. Place in a brown glass bottle and store in a refrigerator.

(d) Sodium arsenite—weigh 1 g. into a 100-ml. volumetric flask and make up to volume with water.

(e) Chromotropic acid—add 600 ml. of reagent sulfuric acid to 300 ml. of water and cool. Dissolve 1 g. of chromotropic acid in 200 ml. of water, add 800 ml. of sulfuric acid solution, and cool. Store in a brown glass bottle; prepare fresh every 2 to 3 wk.

(f) Monoglycerides. Analyses of the commercial monolaurin employed in this study by the method of Martin (10) gave values in excess of 100% when calculated as the monoglyceride. When an ether solution of an aliquot of this product was washed with water to remove glycerol, the most likely contaminant, subsequent analysis indicated a monolaurin content of approximately 84%. Analyses of the monopalmitin and monoolein samples gave values of 94 and 91%, respectively, and these values were not altered by the washing technique. Since monobutyrin is water-soluble, the washing technique could not be employed in the removal of glycerol from this product. Although glycerol was not extracted from the silica-gel column, when applied in aqueous solution following the procedure outlined below, the monobutyrin could not be freed of glycerol by this procedure, because of apparent slight hydrolysis of this monoglyceride after the column treatment. Treatment of ethyl acetate solutions of the crude 1-monobutyrin with 10% sodium sulfate apparently removed at least part of the glycerol, but on short storage of the monoglycerides treated in this manner, periodic acid–oxidizable substances increased, indicating a generation of further adjacent hydroxyl groups. Thus, it appeared that the purity of monobutyrin can be temporarily improved, but that this monoglyceride is apparently relatively unstable.

Extraction procedure. This procedure is essentially the silica-gel method for determining the total free fatty acids in milk, described by Harper *et al.* (8). The buffered layer of silicic acid is omitted from the column and a solvent mixture of 90% chloroform and 10% ethanol is employed. After extracting a sample of milk, usually 10 ml., the solvent is removed under reduced pressure at a temperature not exceeding 50° C. The fat is rinsed with several small portions of chloroform into a volumetric flask of suitable size (10 or 25 ml.), made up to volume with chloroform, mixed thoroughly, and 1 ml. removed for

the estimation procedure. It is necessary to include a blank using water in place of milk.

The extraction and estimation procedures were applied to various samples of normal and rancid milk. Rancid milk was prepared by mixing homogenized and raw milk in equal portions and storing 24 hr. at 4° C. The recovery of monoglycerides from chloroform was studied by adding aliquots directly to the silica-gel and analyzing the extracts for monoglycerides. The extracts were also titrated to determine if hydrolysis had occurred.

Influence of interfering substances. Recovery trials using a 5% lactose solution were made by employing the silica-gel extraction and the estimation procedures, to determine if lactose was extracted. In another series, the extracted fat was washed with water, which was filtered, evaporated, and treated with anthrone to measure the total carbohydrates (6). The analysis for formaldehyde in the extracted fat was made by omitting the oxidant, periodic acid.

Determination of acid degree. The acid degree values on milk samples were determined by the silica-gel method of Harper *et al.* (8).

RESULTS AND DISCUSSION

Estimation procedure. The reliability of the method was determined by analyzing chloroform solutions of monobutyrin, monolaurin, monopalmitin, and monoolein with Martin's and the periodic-chromotropic method. Preliminary trials showed that the use of concentrations of monoglycerides yielding 10 to 30 γ of formaldehyde resulted in highest recovery values for the latter method. The results are summarized (Table 1). The periodic-chromotropic method is more

TABLE 1

Reliabilty of the periodic-chromotropic method for the estimation of 1-monoglycerides in chloroform

	γ Monobutyrin/ml[a]		γ Monolaurin/ml		γ Monopalmitin/ml		γ Monoolein/ml	
	Added[b]	Found[c]	Added	Found	Added	Found	Added	Found
	126	116	135	142	173	171	156	164
	140	129	140	136	174	176	176	183
	153	157	155	152	190	194	180	184
	173	171	156	153	198	191	184	178
	174	162	157	152	244	244	193	204
	190	183	192	199			198	192
	192	192	210	218			200	206
	197	186	213	212			204	195
							258	250
							358	350
							372	384
							389	386
Mean	168.1	162	169.9	170.3	195.8	195.2	241.4	241.8
Standard deviation of a sample		1.9		2.9		0.84		4.8
Per cent recovery		96.4		100.1		99.7		101.2

[a] Contained some glycerol which could not be removed.

[b] Amount added determined from purities obtained by Martin's method (10), which requires at least 3 mg. for analysis.

[c] Periodic-chromotropic method. All figures in the "found" columns are averages of duplicates and are considered as samples.

sensitive than Martin's method, which requires at least 3 mg. of monoglyceride for analysis (10). The slopes of regression lines, calculated from values obtained from each of the four monoglycerides by the periodic-chromotropic method, and the values obtained by Martin's method, were not significantly different from one, nor were the intercepts significantly different from zero (17). Since this statistical method is useful for comparison of two methods of analyzing the same material over a range of concentrations, it appears that the two methods yield comparable results in the ranges tested. The standard deviations in Table 1 were calculated from the differences between duplicates; thus, are a measure of laboratory precision.

Mattson *et al.* (12) found that when determining 1-monoglycerides by iodometric titration of excess periodic acid, the concentration of 1-monoglyceride in a lipid should be in excess of 6% to obtain meaningful results. In the present work, recoveries from purified butterfat were found to be quantitative when the monoglycerides were present at the 0.1 and 1% levels, as shown in Table 2.

Both Martin (10) and Brokaw *et al.* (2) employed the same procedures for isomerizing 2-monoglycerides to 1-monoglycerides. To remove the perchloric acid after isomerization, Martin washed the chloroform-monoglyceride solution five times with water. Brokaw *et al.* simply increased the water content of the periodic acid solution from 5 to 20% and allowed the water to act as a quench. When used with the fat from rancid milk, Martin's washing procedure produces extremely stable emulsions, which are avoided with the technique of Brokaw *et al.* (2). The analyses for 1- and 2-monoglycerides on the digested cottonseed oil indicated that both methods yield the same results. However, when milk fat was analyzed, the washed material always had a lower total monoglyceride content than the unwashed fat. The factor 1.15 allows for incomplete isomerization (2, 10).

Extraction procedure. Extraction of monoglycerides involves the same problems as the recovery of total free fatty acids from milk, plus the necessity

TABLE 2

Detection of 1-monoglycerides at 1.0 and 0.1% levels in purified milk fat by periodic-chromotropic method

	γ Monobutyrin		γ Monolaurin		γ Monopalmitin		γ Monoolein	
	Added	Found	Added	Found	Added	Found	Added	Found
1% level, 200 γ per 20 mg. fat	185	178	175	172	213	207	224	217
	182	172	204	205	197	202	179	178
	182	177	204	209	197	196	179	180
	182	173	204	203	197	193	179	178
	182	182	204	205	197	198	179	180
	182	183			197	199	179	180
Mean	182.5	177.5	198.2	198.8	199.7	199.2	186.5	185.5
Av. % recovery		97.3		100.3		99.7		99.5
0.1% level, 200 γ per 200 mg. fat	144	125	197	196	167	158	201	202
	182	174	204	208	197	208	179	175
	182	183	204	207	197	200	179	177
	182	180	204	194	197	191	179	184
	182	182	204	207	197	198	179	177
	182	181			197	206	179	185
Mean	175.7	170.8	202.6	202.4	192	193.5	182.7	183.3
Av. % recovery		97.2		99.9		100.8		100.3

for avoiding the inclusion of lactose or glycerol. Since the silica-gel method efficiently recovers short-chain fatty acids, a similar recovery of short-chain monoglycerides is implied. The buffer layer used in the extraction column in the original method was omitted without changing the results. A solvent mixture of 90% chloroform–10% ethyl alcohol replaced the original solvent of 95% chloroform and 5% butyl alcohol, because of its greater polarity and ease of evaporation.

Recovery trials from milk were attempted with water-soluble 1-monobutyrin. The commercial material was found to be impure containing, among other compounds, glycerol. The presence of glycerol did not influence the analysis of 1-monobutyrin in chloroform, except to yield a high apparent purity. However, when milk containing 1-monobutyrin was extracted, the recoveries were low, apparently due to the retention of glycerol in the silica-gel column. Trials with aqueous solutions of glycerol showed that it could not be detected in the extracts from the silica-gel column. Quinlin and Weiser (15) recently have shown that glycerol remains strongly adsorbed on a silica-gel column. When chloroform solutions of the monoglycerides were added directly to the silica-gel columns, no hydrolysis or isomerization was evident. The recovery was always close to 100%, except in the case of monobutyrin. Attempts to emulsify the other monoglycerides into milk with a Waring blendor also failed, because of the extreme stability of the foam produced.

Lactose, when oxidized by periodic acid, yields 2 *M* of formaldehyde (5). The trials with 5% lactose solutions, and determinations of carbohydrate in the extracted fat with anthrone, showed that there was no interference from this source. The unoxidized controls did not contain formaldehyde.

Mixtures of fresh raw and homogenized milk were extracted immediately and after 24 hr., and the monoglyceride contents estimated. These data, showing the increase in acid degree and in monoglyceride content after 24 hr. of refrigerated storage, are presented (Table 3). With an acid degree value of ten, theoretically there could be 5 m*M* of monoglyceride present. The data in Table 3 show much lower total monoglyceride contents. If milk lipases follow the same pathway of hydrolysis as pancreatic lipase, then some 2-monoglycerides

TABLE 3

Increases in acid degree and monoglyceride content of raw and homogenized milk mixtures (1:1) after 24 hr. at 4° C.

Acid degrees [a]	1-monoglyceride	2-monoglyceride
	(mM/100 g. fat)	
8.55	0.36	Not run
10.72	0.53	Not run
10.70	0.86	Not run
10.85	0.42	Not run
8.67	0.32	Not run
12.73 [b]	0.72	0.89
11.61 [b]	0.71	0.99
11.42	0.68	0.80
11.24	0.43	0.91
12.95	0.63	0.57

[a] Silica-gel method.
[b] Aged 48 hr.

from which 1-monoglycerides arise by isomerization should be present, plus a relatively large amount of diglyceride and little or no glycerol (11, 12). The relatively large amount of free fatty acids may be accounted for as having been hydrolyzed from triglycerides to yield diglycerides and, thus, may be an indirect indication of the presence of diglycerides. However, the possibility of incomplete extraction of monoglycerides from milk still exists. The large variation in 1-monoglyceride content of the three samples with almost the same acid degree value possibly may be accounted for by differences in degree and/or rate of lipolysis, and by differences in the rate of isomerization of the 2-isomer to the 1-isomer. An analysis for 1-monoglyceride alone may yield variable results, since both the rate and extent of isomerization may vary (10, 12).

Thirty-one samples of normal pasteurized whole milk contained none to traces of monoglycerides when 10-ml. portions were analyzed.

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FREE FAT IN FOAM-DRIED WHOLE MILK

A. TAMSMA, L. F. EDMONDSON, AND H. E. VETTEL
Eastern Utilization Research and Development Division, USDA,
Washington, D. C.

SUMMARY

Concentrates of 50% T. S. were foamed and dried in a vacuum shelf drier, and comminuted through a 20-mesh screen. Methods were developed for determination of free fat (by extraction with carbon tetrachloride) and of that fat readily separated by centrifugation of the reconstituted product.

Large increases in free fat (from 10 to over 90% of the total fat) resulted from aging and lactose crystallization of the concentrate. This effect was reversed by heating immediately before drying. Free fat decreased slightly with increasing homogenization pressure. Free fat contents less than 10% of the total fat were obtained by proper control of these factors.

Dispersibility of the dry milk decreased slightly with increase in free fat. The fat rise under centrifugal force in reconstituted milk was not affected by variation in free fat to about 50%. At high free fat levels, part of the fat was readily separated by centrifugation. Free fat increased with decrease in particle size. A fraction obtained by comminution through a 40-mesh screen, followed by removal of the finest particles by sieving on a 60-mesh screen, was similar in free fat, more dispersible, and much higher in bulk density when compared to the product obtained by comminution through a 20-mesh screen.

The fat in dried whole milk which is extracted by carbon tetrachloride was designated as free fat by Holm *et al.* (5). It was considered as "fat not protected by a protein film." In spray-dried milks the free fat increased slowly with increase in the total fat. Above 24% total fat a steep rise in free fat occurred. Homogenization decreased the amount of free fat and improved the keeping quality. King (6) concluded from microscopic examination of reconstituted milks that free fat in dried milks is demulsified fat. Carbon disulfide, ethyl ether, and carbon tetrachloride were found by Lampitt and Bushill (8) to extract similar quantities of fat from milk powder. They used carbon disulfide as a standard solvent for extraction. The free fat varied from 3 to 14% of the total fat in spray-dried milks, and from 92 to 96% in roller-dried milks. It varied from 58 to 92% in freeze-dried milk according to Nickerson *et al.* (10), but was reduced to from 35 to 75% by homogenization. In spray-dried milks from the same concentrates it varied from 12 to 19% of the total fat. Freeze-dried whole milks were difficult to reconstitute, due to the presence of free fat.

Shipstead (13) found that the free fat was oxidized first during storage of spray-dried milk, and concluded that the rest of the fat is protected from oxidation by solids-not-fat. King (6) stated that part of the free fat is on the surface of dried milk particles, thus making them more hydrophobic and less wettable. Coulter *et al.* (3) have shown that the amount and dispersion of fat in dried milk affect wettability. Stone *et al.* (15) demonstrated a sharp decrease in self-dispersion and ease of dispersion of dried whole milk, if solid fat was

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present. Results obtained by Litman and Ashworth (9) indicate that the free-fat content of dried milk may be related to the development of scum during storage. The development of a fat-protein complex was postulated.

The methods of determining free fat are empirical, and the specific properties of the fractions extracted with various organic solvents have not been defined. It is clear, however, that the amount extracted from dried whole milk varies between wide limits, depending upon many factors. The free fat has been associated both with poor dispersing properties and with oxidative deterioration during storage.

The present study was undertaken to obtain more information concerning the free fat in foam-dried whole milk, a new physical form with excellent dispersibility in water (14). This paper deals mainly with the effect of certain factors on the amount of free fat and with the relationship between free fat and dispersibility. Also, information was gathered on the significance of free fat in relation to some other properties of the dried milk.

METHODS

Determination of free fat. After investigating several methods of mixing dry milk with carbon tetrachloride and recovering the extracted fat, the following method was adopted for routine analysis: At room temperature, 50 ml. of CCl_4 is added to 10 g. of dry milk in a 125-ml. glass-stoppered Erlenmeyer flask. The mixture is then shaken for 30 min. on a Burrell wrist-action shaker at an intensity setting of 5. Filtering follows immediately through an 18.5-cm. (Reeve Angel No. 812) folded filter. A 25-ml. aliquot of the filtrate is transferred to an aluminum evaporating dish and the solvent removed first on a hot plate at 135° C., then in a 100° C. vacuum oven. The fat residue is weighed and the weight multiplied by two. The free fat is expressed as a percentage of the total fat in the dry milk.

The reproducibility of this method was found to be 0.45% of the absolute value (95% confidence interval). Variation of temperature of extraction, amount of CCl_4, shaking time, and standing time before filtering did not affect the reproducibility for any given set of conditions. No sharp borderline was found between the free fat and the rest of the fat. The amount of free fat extracted increased with shaking time. A 30-min. period was chosen, since the rate of extraction after 30 min. was much less than in the region from 0 to 30 min. shaking time.

Determination of fat rise under centrifugal force. The method is based on the extent of fat rise in 50 ml. of reconstituted milk by centrifuging and recovering the top 5 ml. Errors due to analysis of an aliquot of an inhomogeneous top layer are prevented by using the whole top layer. First, acetone is added to denature the protein, then the fat is extracted with petroleum ether and weighed after removal of the solvent and drying. The procedure adopted is as follows: 15 g. of dry milk are added to 50 ml. of water in a 170-ml. separatory funnel. After shaking a few strokes to disperse the dry milk, another 50 ml. of water is added and the mixture shaken for 2 min. It is left for 5 min. and drained

slowly from the funnel to remove the scum. Tubes with 50 ml. of the reconstituted milk were centrifuged for from 10 to 40 min. at 1,000 and 2,000 r.p.m. in an International [1] centrifuge with a 16-in. diameter head. Thirty minutes at 1,000 r.p.m. is suitable for routine examination. The lower 45 ml. is carefully removed with a thin siphon tube. The top 5 ml. is first mixed with 10 ml. of acetone and then extracted with 10 ml. of petroleum ether. After centrifuging for 5 min. at 2,000 r.p.m., the top layer is transferred to a Mojonnier fat dish. Extraction is repeated twice, the last time with the addition of 2 ml. of acetone to the petroleum ether to facilitate separation. The fat residue is weighed after removal of solvent by the Mojonnier procedure as described under "free fat," and expressed as a percentage of the total fat in the reconstituted milk.

Further analysis. The dispersibility was determined by the method of Sinnamon *et al.* (14), using a stirring time of 1 min. in water at 40° F. The dried product was tempered to room temperature before dispersing. Total solids in the filtrate were determined by the Mojonnier procedure. Moisture was determined by the toluene distillation method (1), and α-lactose according to Sharp and Doob's procedure (12). Observations of fat globule sizes were made by the Farrell index method (4).

EXPERIMENTAL AND RESULTS

Preparation of foam-dried whole milk. Mixed herd milk was standardized to a solids-not-fat to fat ratio of 2.75 and preheated in a hot water jacketed vat under continuous agitation at 145° F. for 30 min. The milk was condensed at 115° F. to 16° Bé and standardized to 50% T.S. The concentrate was homogenized at 135° F. in a two-stae Manton-Gaulin [1] homogenizer, as specified in each experiment. Nitrogen was incorporated in the concentrate during homogenization, or during a second homogenization at a lower pressure following the first homogenization, to obtain good foaming. Incorporation during homogenization was achieved by adding approximately 20 ml. of nitrogen (at room temperature and 1 atm. pressure) per liter of concentrate through a fine capillary in the line immediately prior to the homogenizer. Homogenization in the presence of nitrogen may decrease homogenization effectiveness. The fat was homogenized without the presence of nitrogen by incorporating the nitrogen during a second homogenization. The concentrate was cooled to 50° F., foamed in a vacuum shelf drier, and dried for 3 hr. at below 1-mm. pressure to about 2.5–3.0% moisture. The temperature of the product was kept below 120° F. Details of the drying procedure were described by Sinnamon *et al.* (14). Dry foams were comminuted through a 20-mesh screen.

FACTORS AFFECTING FREE-FAT

1. *Effect of homogenization.* In Trial A, concentrated milk, prepared as above described, was divided into three lots. These lots were homogenized at

[1] The use of trade names is for the purpose of identification only, and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

0, 2,000, and 4,000 p.s.i. on the first stage and 500 p.s.i. on the second stage for the latter two lots. Nitrogen was incorporated in a second homogenization at 2,000 and 500 p.s.i., for the first and second stages, respectively.

In Trial B, two lots of the same concentrate were homogenized at 1,500 and 3,500 p.s.i. on the first stage, the second-stage pressure was 1,000 p.s.i., and the nitrogen was incorporated during homogenization. The results of both trials (Curves A and B, Figure 1) show a decrease in free fat with increase in homogenization pressure, which is in agreement with the results of Holm *et al.* (5). However, the over-all effect of homogenization was rather small, being about 1 to 2% difference in free fat per 1,000 p.s.i. pressure difference. The effect was probably lowered by an equalizing tendency of the nitrogen incorpora-

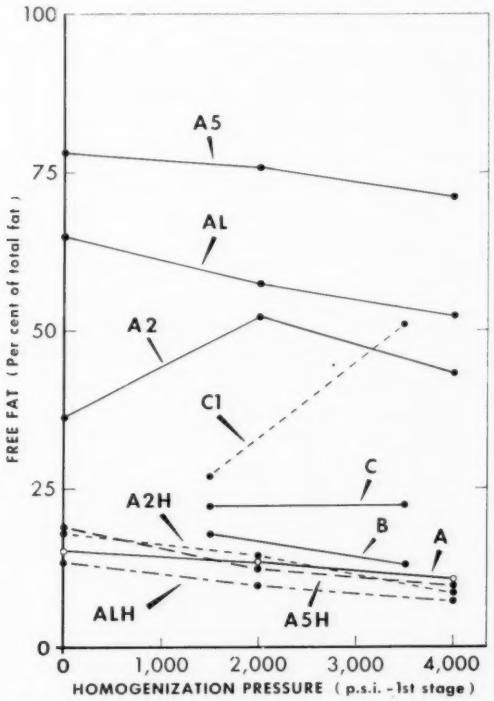

FIG. 1. Effect of homogenization pressure and temperature-time treatment of the concentrate on free fat in foam-dried whole milk.

A = Cooled instantly in pans at 50° F.
A2 = A concentrates held two days at 40° F.
A5 = A concentrates held five days at 40° F.
AL = A concentrates cooled rapidly on surface cooler to 50° F., seeded with 1% lactose, and dried immediately.
A2H, A5H, ALH = A2, A5, AL concentrates heated to 140° F., held for 5 min., and cooled instantly in pans at 50° F.
B = Cooled rapidly on surface cooler to 50° F.
C = Slow cooling in ice-water under stirring.
C1 = C concentrates held one day at 40° F.

tion procedure. Experiments, as in Trial B, where the nitrogen was incorporated during the only cycle through the homogenizer, resulted in excessive knocking when pressures above 1,500 p.s.i. were used. This produced less effective homogenization and was the reason for using a second cycle through the homogenizer for nitrogen incorporation in Trial A. An experiment without incorporation of nitrogen showed a larger effect (from 3 to 10% free fat per 1,000 p.s.i. pressure difference), but these results were of little value because of poor foams and less dispersible products.

The effect of homogenization indicates that superior homogenization will reduce free fat to a lower value. Superior homogenization can be obtained by repeated homogenization at high pressure or centrifugal removal of large fat globules (16). Centrifugal removal of large globules was tried for a 50% T.S. concentrate homogenized at 3,500 and 1,000 p.s.i., for first and second stages, respectively, using a De Laval [1] separator. After separating twice, which resulted in removal of 6% of the fat as cream, the free fat in the dry product was reduced to about three-fourths of that in a control standardized to the same composition.

2. *The effect of heating and aging of the concentrate, and lactose crystallization.* These factors are all inter-related, since the temperature and age of the concentrate greatly affect the degree of lactose crystallization. Each of the three lots in Trial A was further divided into three sub-lots, AL, A2, and A5. AL was cooled on a surface cooler, seeded with 1% lactose, and dried immediately. Sub-lots A2 and A5 were cooled on a surface cooler, then held two and five days, respectively, at 40° F. and dried without further treatment. The marked increase in free fat resulting from these treatments at all homogenization pressures is shown by comparing Curves A2, AL, and A5 with Curve A (in Figure 1). The complete reversibility of the effect of added lactose crystals, and aging of the concentrate, is shown by Curves ALH, A2H, and A5H. These concentrates were the same as AL, A2, and A5, respectively, except they were heated to 140° F., held for 5 min., and cooled immediately to 50° F. just prior to foam-drying. These results strongly indicate a relationship between crystallized lactose in the concentrate and free fat in the dried milk. A five-day-old concentrate (A5, Figure 1) contained 74% of the lactose in α-form, as determined by Sharp and Doob's (12) method. Heating (A5H, Figure 1) lowered this percentage to 37. The equilibrium level without crystallization is 37%.

The effect of lactose crystallization on free fat can supersede the effect of homogenization, as demonstrated by Trial C in Figure 1. A batch of concentrate, prepared as described, was divided into two lots. These lots were homogenized at 1,500 and 3,500 p.s.i. on the first stage, and 1,000 p.s.i. on the second stage, and cooled slowly. Each of the two lots was divided into two sub-lots, C and C1. Sub-lots C were dried immediately, C1 after one day of holding at 40° F. No significant increase in α-lactose was found in these samples, except for the sample C1, homogenized at 3,500 p.s.i., which had 46% of the lactose in the alpha form. Correspondingly, the free fat was highest in this same sample, which reversed the regular sequence: lower free fat with higher homogeni-

zation pressure. The same trend was already present in the C samples, as indicated by Curve C, which was almost horizontal instead of parallel to Curve B. Curve A2 (Figure 1) shows a similar behavior below 2,000 p.s.i. Lactose crystallization apparently had proceeded more in some samples by chance, due to conditions such as the presence of nuclei etc.

The effect of rate of cooling of the concentrate on free fat content is indicated by the results of Samples A, B, and C (in Figure 1). Slower cooling, which favors lactose crystallization, corresponded with higher free fat. No significant difference in α-lactose was found in these samples by Sharp and Doob's method.

Figure 2 shows the relationship between excess α-lactose and free fat, established with data collected from 12 samples, processed identically except for difference in seeding with lactose. A regression line of $Y = 18.29 + 2.50\,X$ was obtained for this relationship (Y = per cent free fat and X = excess α-lactose), with a coefficient of correlation of +0.82 ($P = <0.01$). The standard error is 9.27. The excess of α-lactose is expressed as the difference between the percentage of the lactose in the α-form and the equilibrium value (37%). The regression coefficient, 2.50, shows that relatively small increases in excess α-lactose resulted in large increases in free fat. The excess α-lactose, however, may not represent the crystallized lactose, since Sharp and Doob's method yields the total α-lactose. If crystallization proceeded more rapidly than the shift from β- to α-lactose while holding the concentrate, or during drying, the amount of crystallized lactose would be greater that the excess α-lactose (as shown in Figure 2). It will be noted that the free fat content for 0.0% of excess α-lactose was 18.29, which is considerably higher than the minimum values from Figure 1. This indicates that other factors, including an indeterminate amount of crystallized

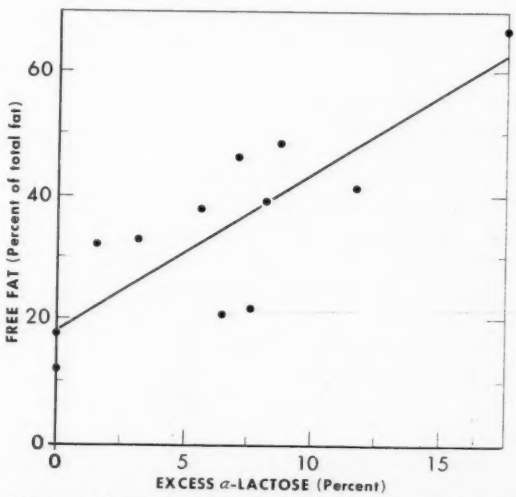

FIG. 2. Relationship between excess α-lactose and free fat.

lactose, also may affect the free fat content. Choi (2) pointed out that variations in free fat may be affected by changes in the proteins.

3. *The effect of particle size.* A concentrated milk was homogenized at 3,500 and 1,000 p.s.i. for first and second stages, respectively. Nitrogen was incorporated during a second cycle through the homogenizer. The dry foam was comminuted through an 8-mesh screen, mixed to a uniform batch, and divided into Lots 1, 2, 3, and 4 (see Table 1). The first lot was further divided into three fractions as follows: Fraction 1 was used as such. The remainder was sieved on a 20-mesh screen into coarse and fine fractions, 1c and 1f, respectively. The second lot was comminuted through a 20-mesh screen and then further divided into three fractions. One-third (Fraction 2) was used as such, and the remainder sieved on a 40-mesh screen into coarse and fine fractions, 2c and 2f, respectively. Similar fractions were obtained with the third lot, using 40- and 60-mesh screens. The fourth lot was comminuted through a 60-mesh screen to give Fraction 4. The data in Table 1 shows an increase in free fat with decrease in particle size (and increase in surface area). These results indicate that free fat is present on the surface of the particles. This was further evidenced by fluorescence microscopy (7) of dry milk comminuted through a 60-mesh screen. The fat appeared as a layer around the particles.

The bulk density (tamped to a constant volume) increased with decrease in particle size resulting from comminution through a higher mesh screen [compare Fractions 1, 2, 3, and 4 (Table 1)]. Fine fractions varied somewhat in bulk density as compared with the original fraction in each series (compare 1 and 1f, 2 and 2f, 3 and 3f). Fractions obtained by sieving were much lower in bulk density than fractions obtained by comminuting through screens of the same size (1f–2, 2f–3, 3f–4). Some difference in form or size distribution of the particles, not reflected in the free fat, must be responsible. The bulk densities of the coarse fractions obtained by sieving in each series were not significantly different from those of the original fractions (compare 1c and 1, 2c and 2, 3c and 3, respectively). Coarse fractions, representing about 70% of the total, were readily dispersible. The dispersibilities for Fractions 2 (the standard product), 2c, and 3c are 88.5, 93.7, and 94.2%, respectively.

TABLE 1

Free fat and bulk density of dry whole milk fractions differing in particle size

Fraction No.	Fractionating procedure		Bulk density (*g. per ml.*)		Free fat (*% of total fat*)
	Comminuted mesh	Sieved mesh	Loose	Tamped	
1	8	no	0.09	0.12	12.6
1c	8	20 coarse	0.09	0.12	7.7
1f	8	20 fine	0.11	0.14	16.7
2	20	no	0.22	0.32	16.7
2c	20	40 coarse	0.25	0.31	12.7
2f	20	40 fine	0.20	0.39	25.2
3	40	no	0.31	0.45	23.7
3c	40	60 coarse	0.37	0.48	17.4
3f	40	60 fine	0.22	0.37	34.7
4	60	no	0.31	0.48	37.6

Utilization of coarse fractions may open a way to obtain good products with higher bulk density (compare 3c and 2, Table 1). The procedure would be comminution through a screen finer than 20 mesh and removal of the finest particles by sieving on a yet finer screen. Sinnamon *et al.* (14) found the dispersibility of powders comminuted through a 40-mesh screen to be lower than those comminuted through a 20-mesh screen.

The effect of comminution temperature. In the standard procedure the foam was comminuted at about 120° F. as it came from the drier with the fat in the liquid state. Four drying runs were made in which half of the foam was held overnight at 40° F. and then comminuted at 40° F. The other half was comminuted immediately by the standard (control) procedure. The free fat and dispersibility of the products comminuted cold are expressed as percentages of the corresponding control values. The free fat varied from 92 to 115%, with an average of 103%; the dispersibility varied from 105–114%, with an average of 108%. The data do not indicate a trend for the free fat, but a slight improvement in dispersibility resulted from the low temperature comminution.

Melting point of the fat. Recombined whole milk concentrates with 50% T.S. were made by homogenizing mixtures of fat and skimmilk concentrate three times at 140° F., using 4,000 and 500 p.s.i. for the first and second stages, respectively. Dispersion of the fat as determined by microscopic observation was similar to that obtained to that obtained by homogenization of whole milk concentrates of 50% T.S. in the usual manner. Three fats were incorporated: whole milk fat, a fraction which was liquid at room temperature, and a more solid fraction remaining from milk fat, after removal of the liquid fraction. The resulting concentrates were processed by the standard procedure. The dry products had properties similar to those of regular foam-dried whole milk. The free fat contents and dispersibilities of the products are expressed as percentages of the values of the corresponding dry milks with whole milk fat. Five sets of dry milks were compared, each with a whole milk fat control and samples with liquid and solid milk fat fractions. The free fat content of the samples containing liquid fat varied from 71 to 154% (average, 111%) of the control; the dispersibilities from 101 to 106% (average 103%). The free fat in those containing solid fat varied from 86 to 166% (average, 132%) of the control; the dispersibilities from 95–101%, with an average of 98%. The data do no indicate trends for the free fat. The dispersibility of the samples containing liquid fat are slightly higher than those containing solid fat. This effect is similar to that reported by Stone *et al.* (15), who showed that milk powders are more easily dispersible at temperatures above the melting point of the fat.

Effect of drying temperature. Drying in the vacuum shelf drier for 6 hr. at 80° F., 4 hr. at 120° F., or 3 hr. at 160° F. shelf temperature did not change the amount of free fat. Drying at 160° F. caused some decrease in dispersibility.

Effect of storage. The initial free fat and dispersibility data of 27 air-packed samples were compared with the data obtained after from 3 to 5 mo. of storage

at room temperature. The samples included the variations in processing previously discussed. The results after storage, expressed as percentages of the original values, varied from 80–115 (average of 105) for the free fat, and from 87 to 109 (average of 102) for the dispersibility. No trends due to storage are indicated by these data. In microscopic observations of Whittenberger (17), no increase in free fat was observed on storage.

Although the experiments reported herein were not designed for taste analysis, some preliminary organoleptic results were obtained. The stored samples developed tallowy flavors. Improved keeping quality was indicated for samples high in crystallized lactose (also high in free fat content), and for samples prepared from aged or lactose-seeded concentrates which were reheated to 140° F. just prior to drying. The effect of lactose is similar to the results reported by Sharp (11). The organoleptic results were obtained by five judges, using a paired comparison preference method of taste testing. Further studies of the effect of heat treatment, and of lactose crystallization in the concentrate, are currently in progress.

Relationship between free fat and dispersibility. The relationship between free fat and dispersibility for 92 samples of foam-dried whole milk is shown (Figure 3). The equation of the regression line is $Y = 97.26 - 0.111X$, the correlation coefficient is $-.54$, and the standard error of estimate is ± 2.54 ($P = <0.01$). The data show that the dispersibility decreased only about 0.11% for each 1.0% increase in free fat. Although the data for this group of samples were combined from many experiments which included variations in several processing steps (heat treatment, homogenization pressure, lactose crystallization, particle size, etc.), the regression coefficient is significant at the .01 level ($P = < .01$).

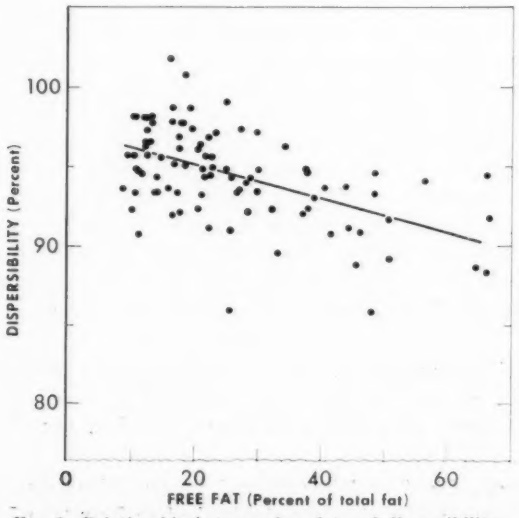

FIG. 3. Relationship between free fat and dispersibility.

Linear regression analysis of 12 of the samples, from experiments in which the only variable was in seeding with lactose, gave a regression equation of $Y = 97.19 - 0.118X$ (approximately the same as for the combined data), a correlation coefficient of $-.71$, and a standard error of ± 1.91 ($P = < .05$).

Fat rise under centrifugal force at different free fat levels. Fat rise was determined in five samples of reconstituted foam-dried milks selected for free fat difference. Results (Figure 4) were about the same at 1,000 r.p.m., except for Sample 3, which showed much more fat rise. At 2,000 r.p.m. (prime series), the extent of fat rise correlated with the size of the fat globules (homogenization effectiveness) rather than with difference in free fat. The data (Figure 4) show that the fat rise under centrifugal force after reconstitution is not affected significantly by the difference in free fat from 26.7–50.6%, as presented in Samples 1, 2, 4, and 5. Variation in this range is probably caused by differences in

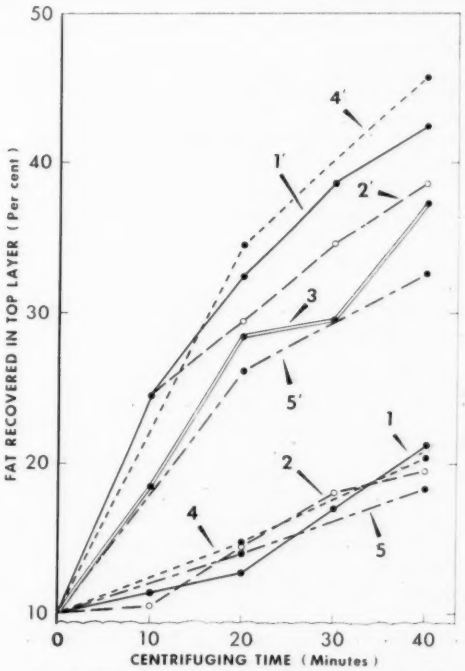

FIG. 4. Fat rise in reconstituted foam-dried milks.

No.	Homo. pressure (*p.s.i.*)	Free fat (%)
1	2,500	29.8
2	2,500	44.2
3	2,500	92.0
4	1,500	26.7
5	3,500	50.6

Plain series centrifuged at 1,000 r.p.m.
Prime series centrifuged at 2,000 r.p.m.

the dry particles, such as the presence or absence of cracks affecting solvent penetration. At very high free fat levels the fat rise under centrifugal force shows major increase (Sample 3), indicating a change in the state of the fat.

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STUDIES ON STARTER METABOLISM. IV. EFFECT OF VARIOUS SUBSTRATES ON THE FORMATION OF ACETOIN BY A MIXED STRAIN STARTER CULTURE[1,2]

W. G. MIZUNO[3] AND J. J. JEZESKI
Department of Dairy Husbandry, University of Minnesota, St. Paul

SUMMARY

The mechanism and the source of carbon for the formation of acetoin by a mixed culture consisting predominantly of *Streptococcus cremoris* and *Leuconostoc* species have been studied, using both a reconstituted milk and a dialyzed milk medium.

Of the various metabolites added to the dialyzed medium, only citric, oxalacetic, pyruvic, and *alpha*-keto butyric acids stimulated the formation of ketol; the *alpha*-keto butyric acid gave rise to a ketol tentatively identified as 3-hydroxy-2-pentanone. Acetaldehyde, isobutyraldehyde, and crotonic acid inhibited acetoin formation. The tricarboxylic acid cycle intermediates, malic, succinic, *cis*-aconitic, and *alpha*-keto glutaric acid, had no effect.

Using the dialyzed milk medium, it was shown that the addition of glucose alone did not result in the formation of acetoin, regardless of the initial pH of the medium. When citric acid alone was added, only a trace of acetoin was formed at pH 7.0, but a considerable amount was formed at pH 5.0. When glucose and citric acid were added, a considerable amount of acetoin was formed, regardless of the initial pH.

Studies with various metabolic inhibitors indicated that, in general, the acid formation was more readily inhibited than was acetoin formation. At an inhibitor concentration such that a partial inhibition of the formation of acetoin was manifested, the addition of more citric acid overcame the inhibition.

Alpha-acetolactic acid decarboxylase activity was demonstrated in the ground cells of a *Leuconostoc* isolate obtained from the mixed culture.

Although the mechanism of formation of acetoin and diacetyl by the bacteria belonging to the genus *Leuconostoc*, and more particularly in cultures containing strains of the *Streptococcus lactis* group and *Leuconostoc* growing in association with each other, has been studied by several workers (2, 3, 4, 6, 7, 15, 17, 19), the carbon source(s) and the mode of transformation have not been conclusively demonstrated. Some workers believe that citric acid is the carbon source for the formation of acetoin (4, 5, 15), whereas others believe that lactose is the carbon source (2, 19). The milk media used by most of these workers contained both citric acid and lactose and either one, on the basis of currently accepted pathways, could serve as the carbon source. The stimulation of acetoin formation upon the addition of citric acid to a complex medium already containing this acid can not be accepted as proof that citric acid is the carbon source for the acetoin.

In other species, the immediate precursor has been shown to be *alpha*-acetolactic acid (10, 13, 20), with pyruvic acid as the precursor for the aceto-

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[3] Present address: Economics Laboratory, Inc., St. Paul, Minnesota.

lactate. The mechanism for the formation of acetoin from citric acid is less well understood.

MATERIALS AND METHODS

Bacteriological. The starter culture (WC) contained both lactic streptococci and *Leuconostoc* species and has been maintained in the Dairy Bacteriology laboratory, University of Minnesota, for a number of years.

This culture was transferred routinely at 24-hr. intervals in reconstituted nonfat dry milk solids (NDMS), at the rate of 100 g. in 1 liter of distilled water and using a 5% inoculum, followed by incubation at 21° C.

For some fermentation studies, a reconstituted NDMS medium was prepared by dispersing 100 g. of NDMS in 900 ml. distilled water, to allow for dilution due to addition of supplementary metabolite solutions. This medium and that used for routine transfer purposes were autoclaved at 17 p.s.i. steam pressure for 15 min.

For other fermentation studies, a dialyzed milk medium was prepared in the following manner: Concentrated reconstituted NDMS (180 g. in 600 ml. distilled water) in Visking cellulose tubing was dialyzed against distilled water at 3–5° C. for four days. The final dialyzed milk medium contained milk dialysate plus the various kinds and amounts of growth factors and salts found to be essential for the growth of *S. lactis* by Niven (14), and the various metabolites under study, such that the total volume was 250 ml. and the resulting medium contained the equivalent of 100 g. NDMS in 900 ml. water. This medium was autoclaved for 5 min. at 15 p.s.i. steam pressure, and the asparagine solution, glucose, and/or other metabolite(s) were added after the milk was cooled to 20° C. in a water bath. The asparagine and other unstable metabolites were sterilized by use of a Seitz filter. Solutions of stable metabolites were heated in a boiling water bath for 10 min.

A single lot of low-temperature spray-dried NDMS was used throughout this study. An analysis of the reconstituted milk (100 g. in 900 ml. water) indicated that no acetoin, 0.21 g. citric acid, 5.8 g. lactose, less than 0.005 g. glucose, and less than 0.006 g. galactose were present per 100 g. of reconstituted milk. The milk, after dialysis, contained less than 0.003 g. citric acid and less than 0.002 g. lactose per 100 g. of reconstituted milk.

Chemical. Acetoin and diacetyl were determined together because preliminary experiments had indicated that acetoin was the principal end-product, with only several per cent of the total existing as diacetyl. The method of van Niel (18), as modified by Michaelian *et al.* (12), was used for determining acetoin, and the method of Michaelian (12) was used for determining diacetyl.

Glucose and lactose were assayed according to the method of DuBois *et al.* (5). On an unknown mixture such as the NDMS, the carbohydrates were first separated by paper partition chromatography (9). Where only a single carbohydrate was present, the sample was first deproteinized with an equal volume of 10% trichloroacetic acid and clarified either by filtration through a glass wool plug or by centrifugation.

Citric acid was determined by the colorimetric method of Babad and

Shtrikman (1). The milk sample was first deproteinized with trichloroacetic acid and clarified as for the carbohydrate assay.

The fermentation vessel consisted of a 500-ml. Erlenmeyer flask fitted with a center well and rubber stopper, to which was attached a Bunsen valve, to permit the entrance of air into the vessel but to prevent the escape of gases to the outside. The carbon dioxide was collected in the center well, using 8 ml. of 20% CO_2-free KOH solution. The carbon dioxide collected was assayed gravimetrically in the form of barium carbonate.

The total acid in the culture was determined by titrating a 9.0-ml. aliquot to the phenolphthalein end-point with freshly standardized 0.1 *N* NaOH solution, after adding about 75 ml. of distilled water to the assay sample.

EXPERIMENTAL RESULTS AND DISCUSSION

Characterization of culture. Fifty isolates from a 20-hr. culture were obtained by conventional plating procedures, using a yeast extract–tomato juice medium and characterized on the basis of reaction in litmus milk, production of ammonia from peptone, growth in media containing 4.0 and 6.5% sodium chloride, growth in a medium adjusted to pH 9.2, growth at 10, 40, and 45° C., growth in milk containing 0.1% methylene blue, survival of exposure to 60° C. for 30 min., and acetoin and total acid in reconstituted NDMS. Smears were made using Loeffler's methylene blue stain, to check on morphology. Twenty-nine of the 50 isolates were found to form acetoin and produce low acidity; the remaining 21 formed no acetoin but produced the acidity expected of the lactic streptococcus group. Each group was relatively homogeneous. The failure of the lactic group to form ammonia from peptone, to grow at 40° C., to grow in a medium of pH 9.2, and for the majority of them to grow in a medium containing 4.0% sodium chloride indicated that they were predominatly *Streptococcus cremoris*. The ability of the acetoin producers to produce carbon dioxide from dextrose indicated that they were of the genus *Leuconostoc*, rather than citrate-fermenting streptococci.

Production of acetoin and acid by Culture WC as a function of time of incubation. Total acetoin and diacetyl commonly are determined together. However, a large part of this total is acetoin and this ratio probably depends upon growth conditions and the culture used. When the mixed culture was grown in an NDMS medium and incubated for 20 hr. at 21–22° C., the ratio of the diacetyl to acetoin was found to be 1.8 to 27 mg. per 200 ml. of this culture; thus, 94% of the total acetoin-diacetyl existed as acetoin, and acetoin appeared to be the metabolically important end-product. Consequently, in the following, as well as in most of the subsequent studies, assays were made for the combined acetoin and diacetyl.

Production of acetoin and acid as a function of time of incubation was determined on the NDMS medium, NDMS plus 0.5% added glucose, and NDMS plus 0.3% added citric acid. The results presented in Table 1 show that under these experimental conditions, the acidity approached a maximum in 24 hr. of incubation at 21° C. with the NDMS. The acetoin, however, reached a maximum

TABLE 1

Development of acetoin and acid by Culture WC as a function of time of incubation at 22° C.[a]

	Incubation at 22° C.	Final pH	Final acidity	Acetoin 200 ml. culture
	(hr.)		*(%)*	*(mg.)*
Milk alone	0	6.5	0.14	....
	4	6.3	0.15	2.4
	8	5.9	0.21	5.1
	12	4.9	0.51	34.3
	14	4.5	0.63	29.5
	24	4.3	0.73	26.7
Milk plus 0.5% glucose	0	6.5	0.13	....
	4	6.4	0.15	trace
	8	5.9	0.21	4.9
	12	4.9	0.51	36.0
	18	4.4	0.71	29.0
	24	4.3	0.75	29.4
Milk plus 0.3% citric acid	0	5.0	0.46	....
	4	5.0	0.46	2.1
	8	5.0	0.46	15.0
	12	5.0	0.56	71.0
	18	4.6	0.70	85.6
	24	4.5	0.72	80.8

[a] Medium: reconstituted NDMS. Initial acidities are indicated by the zero-time titration.

in 12–18 hr. and there was a slight decrease thereafter. The addition of glucose to the NDMS medium had little, if any, effect either on terminal acidity or on acetoin production. The addition of citric acid resulted in a marked increase in the production of acetoin, but did not materially alter the terminal acidity. These data indicate that in the NDMS medium there is a surplus of the carbohydrate, but apparently not of the citric acid. Other experiments in which the terminal concentrations of carbohydrate and citric acid were determined substantiated this conclusion.

Effect of various metabolites on the fermentation products. Various metabolites, possible precursors and intermediates, were added to the NDMS or the dialyzed milk medium to study the mechanism involved in the formation of acetoin. In earlier studies the NDMS medium was used; whereas, in the later studies, the dialyzed milk medium was used.

Table 2 is a summary of the effect of adding various metabolites to the NDMS medium. The control to which no additions were made shows an appreciable development of acetoin and acid due, apparently, to the presence of citric acid and lactose in the NDMS. The last column in the table shows the production of acetoin above or below the control fermentation. Of the 15 compounds added, only citrate and pyruvate produced any significant stimulation of acetoin formation. Acetaldehyde at a concentration of 0.05% (0.011 *M*) produced a definite inhibitory action toward the production of both acetoin and acid; this would suggest general inhibition of growth. None of the sugars added, namely, glucose, lactose, galactose, and sucrose, had any significant effect on the formation of acid or acetoin, probably because lactose was already present in a sufficient amount.

TABLE 2

Effect of adding various metabolites and salts to a milk medium on the formation of acid and acetoin by Culture WC [a]

Additions	Concentration of metabolite	Final pH	Final acidity	Acetoin	
				Per 200 ml. culture	Difference sample—control
	(%)		(%)	(mg.)	
None (control)	...	4.4	0.76	28	0
Glucose	0.50	4.3	0.75	27	− 1
Lactose	0.50	4.4	0.72	29	1
Galactose	0.50	4.4	0.68	30	2
Sucrose	0.50	4.5	0.78	26	− 2
Citric Acid	0.30	5.8	0.84	80	52
Na citrate	0.30[b]	5.2	0.66	82	54
Na pyruvate	0.077	4.4	0.72	50	22
Lactic Acid	0.15	4.4	0.69	29	1
Malic Acid	0.15	4.4	0.67	25	− 3
Succinic Acid	0.17	4.5	0.83	27	− 1
Ascorbic Acid	0.15	4.4	0.72	27	− 1
Na acetate	0.20	4.7	0.84	28	0
Na glycerophosphate	0.15	4.5	0.72	27	− 1
2/3 Butylene glycol	0.15	4.5	0.80	35	7
Acetaldehyde	0.015	4.4	0.79	30	2
Acetaldehyde	0.05	6.0	0.19	7.6	−20.4
Acetaldehyde	0.15	6.0	0.20	2.6	−25.4
Ethanol	0.30	4.5	0.72	28	0

[a] Incubation temperature and time: 21–22° C. for 20–24 hr. Medium: reconstituted NDMS.
[b] 0.3% as citric acid.

Neither malic nor succinic, two of the tricarboxylic acid (TCA) cycle intermediates, had any significant effect on the formation of acetoin. This failure to stimulate the formation of acetoin might be due either to lack of permeability of these substances or to the absence of the TCA cycle or to the lack of dependence on this cycle. A fourth possibility, namely, that these acids are not the rate-limiting factor (i.e., that they are already present in excess) is unlikely, because the terminal concentration of citric acid has been shown to be extremely low in other experiments and, under these conditions, if the succinic and malic acids were a part of the reaction chain, they ought to be utilized eventually. Because total acetoin rather than the rate of formation was studied, one would expect that when the citric acid is completely utilized the microorganisms would start to use malic or succinic acids for the synthesis of acetoin, if they were intermediates.

Table 3 is a summary of the effect of adding various metabolites to the dialyzed milk medium containing 1.0% added glucose and adjusted to pH 5.0 with 2 *M* orthophosphoric acid. Citric, pyruvic, and oxalacetic acids were the only ones in this series which stimulated the formation of acetoin. *Alpha*-keto butyric acid stimulated the formation of an acyloin similar to but not identical with acetoin, as indicated by the atypical color of the nickel dioxime salt. *Trans*-aconitic, *cis*-aconitic, citraconic, *alpha*-keto glutaric, and *beta*-hydroxy butyric acids had no apparent effect. The failure of some of the TCA cycle intermediates to stimulate the formation of acetoin suggested a possibility that this cycle, as

TABLE 3

Effect of various metabolites added to dialyzed milk on fermentation products produced by Culture WC[a]

Additions	Final pH	Acidity		Acetoin per 200 ml. culture
		Initial	Final	
		(%)		(mg.)
None	4.5	0.55	0.82	Trace[c]
0.15% Citric Acid	4.3	0.69	0.94	42.7
0.15% *Trans*-Aconitic Acid	4.5	0.48	0.71	Trace
0.15% *Cis*-Aconitic Acid	4.4	0.51	0.67	Trace
0.15% Itaconic Acid	4.5	0.51	0.68	Trace
0.15% Citraconic Acid	4.4	0.51	0.63	Trace
0.15% *alpha*-Keto glutaric Acid	4.6	.....	0.60	Trace
0.15% *beta*-Hydroxy butyric Acid	4.7	0.58	0.72	0.1
0.15% *alpha*-Keto butyric acid	5.2	0.50	0.58	20.9[b]
0.077% Sodium pyruvate	4.3	0.56	0.74	15.0
0.10% Oxalacetic Acid	.....	.....	.....	13.4

[a] Medium: dialyzed milk. All flasks had 1.0% added glucose. pH of medium adjusted to 5.0 with 2 *M* H_3PO_4.

[b] An orange-colored nickel glyoxime salt was obtained, in contrast to the red nickel dimethylglyoxime formed with other samples.

[c] Trace is estimated to be about 1 mg. of precipitate or less. Sample was not weighed.

such, was not operative. This was the reason for testing some of the other decomposition products of citric acid, i.e., to determine whether the degradation of citric acid followed another route different from the conventional TCA cycle. In these studies, where the incubation time was relatively long, there are maximum opportunities for permeability and adaptation to take place, so that failure of a compound to stimulate the formation of acetoin may be interpreted to mean that it is not a precursor of acetoin, with somewhat a greater degree of assurance than in resting cell studies.

In other studies (not reported here), the addition of isobutyraldehyde and and crotonic acid inhibited acetoin formation.

A further study of alpha-keto butyric acid utilization. It seemed worthwhile, at least tentatively, to identify the ketol produced in this transformation. Accordingly, the nickel dioxime salt obtained from this fermentation was *(1)* analyzed for its nickel content; *(2)* a spectrogram of the nickel dioxime in the visible region prepared; and *(3)* the 2,4-dinitrophenylhydrazone derivative of the corresponding diketone of the unknown ketol prepared.

A spectrogram of the unknown nickel dioxime, as well as one from a known sample of dimethylglyoxime, was prepared in the region of 325–700 mμ, with the aid of a Model B Beckman spectrophotometer. Since both of these nickel salts were practically insoluble in a number of common solvents tested, the samples were prepared by suspending the nickel salt in a clear lacquer and making a thin, uniform smear on a glass slide 1 by 4 cm., cut to fit the cuvette holder of the spectrophotometer; a blank was made by preparing a film of clear lacquer in a similar fashion. The spectrograms were prepared in the usual manner, by standardizing the instrument with the blank and determining the optical densities of the samples at appropriate wave lengths. The data summarized in Figure 1 indicate that the nickel dimethylglyoxime had a maximum absorption

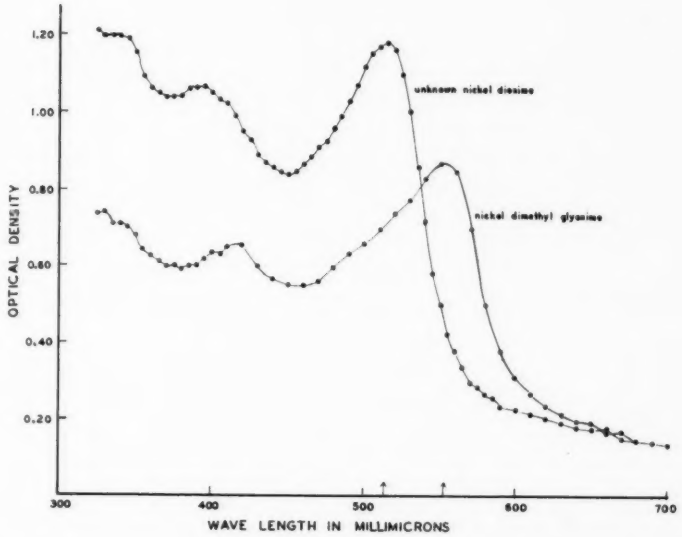


FIG. 1. Spectrograms of nickel dimethylglyoxime and unknown nickel dioxime.

band at about 550 mμ; whereas, the nickel salt of the unknown dioxime had a maximum absorption band at 515 mμ. Furthermore, since the minor maxima at 390 and 415 mμ have peaks which were somewhat different in shape, the data indicate that the two compounds were not identical, and also indicate that there was little or no nickel dimethylglyoxime in the unknown sample, since the peak at 550 mμ, the maximum absorption band for the nickel dimethylglyoxime, was absent from the curve of the unknown sample.

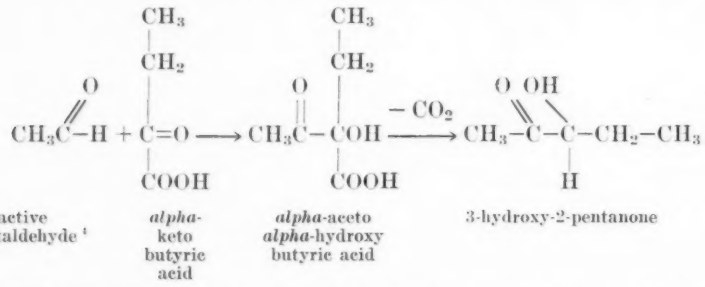
The unknown nickel dioxime was decomposed with concentrated nitric acid, evaporated to dryness to remove the excess acid and the decomposition products(s), and the liberated nickel assayed gravimetrically as the nickel dimethylglyoxime by the conventional method. Twenty-one and four-tenths milligrams of the unknown nickel dioxime yielded 19.5 mg. of the nickel dimethylglyoxime. If the unknown nickel dioxime were the nickel methylethylglyoxime, the expected yield would be 19.5 mg.; if the unknown nickel dioxime were the nickel diethylglyoxime, the yield should be 17.9 mg. of the nickel dimethylglyoxime. These data suggest that the unknown nickel dioxime was probably nickel methylethylglyoxime; and, hence, the unknown ketol formed in the presence of *alpha*-keto butyric acid was 3-hydroxy 2-pentanone rather than acetoin or propanoin, the three most likely products expected to be formed by this fermentation.

The unknown ketol produced in the presence of *alpha*-keto butyric acid was oxidized with ferric chloride and the diketone formed was isolated by steam distillation in a conventional manner. The 2,4-dinitrophenylhydrazone derivative of this diketone was prepared according to the method of Shriner and Fuson (16), and the product was recrystallized from 95% ethanol. The

product had a melting point of 278–283° C. (uncorrected), which is identical to that reported in the literature for the 2,4-dinitrophenylhydrazine derivative of acetylpropionyl (8). The melting points for the 2,4-dinitrophenylhydrazone derivatives of diacetyl and dipropionyl are reported to be 315 and 145–5.5° C., respectively (8, 16).

These studies on the ketol formed in the presence of *alpha*-keto butyric acid tentatively characterize the unknown ketol as 3-hydroxy-2-pentanone.

The 3-hydroxy-2-pentanone apparently is formed in a manner analogous to acetoin. A possible pathway for this formation is suggested below:



A similar pathway with active acetaldehyde and pyruvic acid could give rise to acetoin.

Apparently, the active acetaldehyde precursor, pyruvic acid, can not be replaced by *alpha*-keto butyric acid; otherwise, one would expect propanoin to be formed instead of 3-hydroxy-2-pentanone. The formation of 3-hydroxy-2-pentanone in this fermentation would suggest that a condensation is taking place between the active acetaldehyde and *alpha*-keto butyric acid, with subsequent decarboxylation of the *alpha*-aceto *alpha* hydroxy butyric acid, to form the 3-hydroxy-2-pentanone as indicated in the above scheme.

Why the addition of pyruvic acid or *alpha*-keto butyric acid, but not glucose alone, is able to stimulate a ketol formation is not clear, since some pyruvic acid presumably must be present in the medium from glycolysis. Perhaps a suitable oxidation-reduction balance favorable for the formation of acetoin can not be attained in the absence of added citric, pyruvic, or *alpha*-keto butyric acids. It appears that a relatively high concentration of pyruvic acid is needed for the acetoin to be synthesized; since pyruvic acid is an active key intermediate, this is not surprising.

Effect of varying the glucose and citric acid levels in the dialyzed milk medium on the fermentation products. To study the effect of adding glucose and/or citric acid independently, and without complications due to the presence of citric acid or carbohydrate already present in normal milk, a dialyzed milk medium was used. Since there appears to be some question as to which one of these compounds is the source of carbon for the formation of acetoin, it is important that they be added independently.

[1] The term active acetaldehyde was used by Juni (9) to denote nascent aldehyde.

The data obtained in this study and summarized in Table 4 indicate that in the absence of any added glucose or citric acid, no acetoin and only a very small amount of acid were produced in the dialyzed milk medium. When 2.0% glucose was added, acid but no acetoin was formed. When 0.25% citric acid was added, only a small amount of acetoin was formed, with little or no acid. When glucose was kept constant (1.0%), and the citric acid concentration was varied, the acetoin formed was a function of, but not directly proportional to, the citric acid added. Conversely, when the citric acid was kept constant (0.25%) and the glucose concentration was varied, the acetoin formed was again a function of, but not directly proportional to, the glucose added. The data also show that when 1.0% glucose was added, about one-half to three-quarters of it was utilized.

TABLE 4

Effect of varying glucose and citric acid concentrations on fermentation products[a]

Additions	Final pH	Final acidity	Acetoin per 200 ml. culture	Final citric acid	Final glucose
		(%)	*(mg.)*	*(%)*	*(%)*
None	6.2	0.21	None	.....[b]	.....
2% Glucose	4.6	0.62	Trace	.....	.....
0.25% Citric Acid	6.6	0.20	2.1	.....	.....
2% Glucose plus 0.25 % citric acid	4.8	0.65	64.0	.....	.....
Reconstituted nonfat milk medium	4.6	0.84	35.0	.....	.....
1% Glucose plus 0.062% citric acid	4.6	0.57	7.5	0.01	0.48
1% Glucose plus 0.125% citric acid	4.6	0.60	24.0	0.007	0.41
1% Glucose plus 0.25% citric acid	4.7	0.69	54.0	0.005	0.28
1% Glucose plus 0.50% citric acid	5.5	0.44	89.0	0.007	0.22
0.25% Citric Acid plus 0.03% glucose	6.4	0.18	4.2	0.02	0.020
0.25% Citric Acid plus 0.10% glucose	6.3	0.18	14.8	0.015	0.024
0.25% Citric Acid plus 0.30% glucose	5.9	0.26	54.3	0.010	0.026
0.25% Citric Acid plus 1.00% glucose	4.7	0.64	59.6	0.010	0.24

[a] Medium: dialyzed milk medium with the one exception noted above. pH of medium unadjusted. Citric acid added as trisodium citrate. Incubation temperature and time: 21–22° C. for 20–24 hr.

[b] Indicates that analysis was not made.

Effect of pH of dialyzed milk medium with and without glucose and/or citric acid on the fermentation products. The results of the previous experiments did not show clearly whether the increased formation of acetoin upon the addition of glucose (in the presence of constant level of citric acid) was due to acid or pH effect, or whether glucose itself could have been a specific stimulant. To study this point further, the pH of the medium was adjusted either with lactic or with phosphoric acids. The data in Table 5, which summarizes this study, show that no acetoin was produced by the culture from glucose alone at pH 6.0 or 5.0. However, acetoin was produced in the medium containing citric acid alone, and the amount was very much dependent on the pH of the medium: At pH 7.0, only a trace, at pH 6.0, 18 mg., and at pH 5.0, 51 mg. of acetoin were produced per 200 ml. of culture. In other experiments, where the initial pH was adjusted to 4.5, poor growth and production of acetoin were obtained. Some workers (13) have reported that the optimal pH for the formation of acetoin

TABLE 5

Effect of pH on fermentation products produced by Culture WC in dialyzed milk medium[a]

Additions	pH adjusted with	Initial pH	Final pH	Acidity final	Acetoin per 200 ml. culture	Final citric acid	Final glucose
				(%)	*(mg.)*	*(%)*	*(%)*
None	Lactic Acid	5.0	5.0	0.34	Trace	0.00	.....[b]
1% Glucose	Lactic Acid	5.9	4.5	0.47	0	0.00	0.72
1% Glucose	Lactic Acid	5.0	4.9	0.39	0	0.00	0.92
0.25% Citric Acid	Lactic Acid	7.0	6.6	0.17	Trace	0.13	0.014
0.25% Citric Acid	Lactic Acid	6.0	5.8	0.30	18	0.021	0.022
0.25% Citric Acid	Lactic Acid	5.0	5.6	0.37	51	0.005	0.031
1% Glucose plus 0.25% citric acid	Lactic Acid	5.0	5.0	0.46	65.7	0.005	0.78
1% Glucose	H_3PO_4	6.0	4.4	0.83	0.0	0.006	0.50
1% Glucose	H_3PO_4	5.0	4.4	0.79	0.0	0.00	0.75
0.25% Citric Acid	H_3PO_4	7.1	6.6	0.20	1.2	0.20	0.015
0.25% Citric Acid	H_3PO_4	6.0	6.0	0.42	19.2	0.02	0.022
0.25% Citric Acid	H_3PO_4	5.0	5.5	0.70	42.5	0.006	0.027

[a] Citric Acid added as trisodium citrate. Incubation temperature and time: 21–22° C. for 20–24 hr.

[b] Indicates that no analysis was made.

is about 4.5, using resting cell studies. A distinction must be made as to whether the optimal pH is defined on the basis of the rate or of the total acetoin formed. Cultures containing glucose and adjusted to pH 5.0 had a higher terminal glucose concentration than those adjusted to pH 6.0, suggesting that pH or acidity had a limiting influence on the glucose consumption.

In other studies, where lactose was used in place of glucose, similar results were obtained, indicating that these two sugars are practically interchangeable for these studies.

Effect of various metabolic inhibitors on fermentation pattern. Several metabolic inhibitors, including malonic acid, iodoacetic acid, mercuric chloride, sodium fluoride, sodium azide, and potassium cyanide, were added to the culture grown in reconstituted milk (NDMS), to study the pattern of inhibition. In general, almost without exception, it was found that acid production could be inhibited more readily than could acetoin production.

For the sake of brevity, only the inhibition studies using iodoacetic acid as the inhibitor will be presented. These data, summarized in Table 6, indicate that about 1.0×10^{-5} *M* of iodoacetic acid produced complete inhibition, whereas 3.0×10^{-6} *M* resulted in complete inhibition of acid production and about 50% inhibition of acetoin production. When the formation of acetoin was inhibited, the citric acid was not utilized to any appreciable extent. The addition of citric acid and, likewase, pyruvic acid was found to overcome this inhibition, suggesting that it was a competitive inhibition.

Since iodoacetic acid is known to block the formation of pyruvic acid (in the glycolytic sequence) by inhibiting the 1,3-diphospho-glyceraldehyde dehydrogenase, supplying pyruvic acid as such, or a precursor of pyruvic acid such as citric acid (which is apparently capable of forming pyruvic acid by another

TABLE 6

Effect of iodoacetic acid with and without added citrate and pyruvate on the formation of acid and acetoin by Culture WC [a]

Additions	Final pH	Final acidity	Acetoin per 200 ml. culture	Final citric acid
		(%)	*(mg.)*	*(%)*
None	4.4	0.79	25.0	0.01
1.0×10^{-6} *M* CH_2ICOOH	4.6	0.64	21.6	..... [b]
3.0×10^{-6} *M* CH_2ICOOH	5.7	0.22	14.8	.....
1.0×10^{-5} *M* CH_2ICOOH	6.1	0.17	0.0	.....
3.0×10^{-5} *M* CH_2ICOOH	6.2	0.16	0.0	0.17
1.0×10^{-4} *M* CH_2ICOOH	6.2	0.16	0.0	0.17
3.0×10^{-4} *M* CH_2ICOOH	6.3	0.16	0.0	.....
1.0×10^{-3} *M* CH_2ICOOH	6.3	0.17	0.0	.....
3.0×10^{-6} *M* CH_2ICOOH alone	5.7	0.22	14.8	.....
3.0×10^{-6} *M* CH_2ICOOH plus 0.3% citric acid	6.1	0.21	67.0	0.10
3.0×10^{-6} *M* CH_2ICOOH plus 0.077% Na pyruvate	5.7	0.25	22.0	0.01
3.0×10^{-6} *M* CH_2ICOOH plus 0.15% Na glycerophosphate	5.7	0.26	18.0	0.01

[a] Meduim: reconstituted nonfat milk (contains approximately 0.21% citric acid). Citric acid added as trisodium citrate. Incubation temperature and time: 21–22° C. for 20–24 hr. Initial acidity of milk usually 0.14–0.18%.

[b] . . . . Indicates that the analysis was not made.

route), is expected to overcome the inhibition of iodoacetic acid. This appears to be a reasonable explanation of what happened in these experiments.

The fact that pyruvic acid is a common intermediate in the formation of lactic acid and acetoin would suggest the possibility of a certain degree of interdependence, not only between the two types of bacteria but also in the synthetic pathway of these two compounds. On the other hand, the differential inhibition of the formation of acid and acetoin by iodoacetic would suggest some degree of independence of the two pathways.

Why the addition of citric or pyruvic acids did not increase the production of both the acetoin and acid, if pyruvic acid is a common key intermediate, might be explained by the supposition that a suitable oxidation-reduction balance for the formation of lactic acid did not exist or that the inhibitors preferentially inhibited one type of bacteria and not the other.

Alpha-acetolactic acid decarboxylose activity in ground cells of a Leuconostoc isolate. A *Leuconostoc* species isolated from the mixed culture was incubated in a medium (consisting of 10 g. of yeast extract, 20 g. of glucose, 5.0 g. of tryptone, 30 ml. of canned tomato juice, and 5.0 g. of trisodium citrate pentahydrate made up to one liter with distilled water–reaction unadjusted) for 40 hr. at 21–22° C., and harvested by centrifugation. Excess moisture was removed by blotting with sterile filter paper pad, and 1.0 g. of the resulting cell paste was ground at 0–3° C. into 6.0 ml. of 0.05 *M* potassium phosphate buffer (pH 7.0) in a mortar and pestle with the aid of fine, sterile washed sand. The supernatant containing the crude enzyme was tested for *alpha*-acetolactic acid decarboxylase activity.

Each Warburg vessel contained 2.4 ml. of 0.13 *M* potassium phosphate buffer, pH 5.5, and 0.3 ml. of the crude enzyme; 0.2 ml. of *alpha*-acetolactic acid solution

in the side arm (150 mg. of *alpha*-acetolactic acid per 5.0 ml. of solution), and 0.3 ml. of 40% KOH or water in the center well. Total fluid volume of the vessel was 3.2 ml. The gas phase was air and the temperature was 30° C. The *alpha*-acetolactic acid was prepared according to the method of Krampitz (11).

The results of this study are summarized in Figure 2. The heated enzyme preparation was roughly one-half as active as the unheated enzyme preparation. Although the spontaneous breakdown was undesirably high, the data do indicate that the crude enzyme preparation has a definite *alpha*-acetolactic acid decarboxylase activity. The data substantiate by analogy the scheme for the formation of 3-hydroxy-2-pentanone from *alpha*-keto butyric acid, and lend support to the theory that acetoin is probably formed by way of active acetaldehyde and *alpha*-acetolactic acid from pyruvic acid.

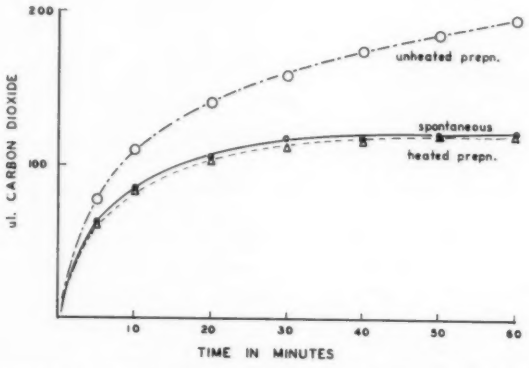


FIG. 2. *Alpha*-Acetolactic Acid decarboxylase activity of ground cells of Isolate No. 12.

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A RAPID SPECTROPHOTOMETRIC METHOD FOR MEASURING CHEESE RIPENING [1]

D. G. VAKALERIS AND W. V. PRICE
Department of Dairy and Food Industries, University of Wisconsin, Madison

SUMMARY

A rapid method for estimating the degree of proteolysis in ripening cheese was studied by measuring absorption of ultraviolet light by a clear, sodium citrate–hydrochloric acid extract of cheese at pH 4.4 ± 0.05; these measurements were closely correlated with the per cent of soluble nitrogen in the cheese extract.

Measurements of the optical density of the cheese from 40 lots of varying ages revealed peaks of all absorption spectra between 274 and 275 mμ. The coefficient of correlation between optical density of the cheese extract and percentage of soluble nitrogen measured in the cheese extract by Kjeldahl was 0.958.

Concentrations of soluble tyrosine and soluble tryptophane were measured spectrophotometrically at optical densities of 270 and 290 mμ. Equations were developed by Vierordt's method of analysis of two component systems for this pair of wave lengths. Relationships between these compounds and percentage of soluble nitrogen in the cheese were linear and highly significant; the coefficient of correlation between soluble tyrosine and percentage of soluble nitrogen was 0.964; whereas, that between soluble tryptophane and percentage of soluble nitrogen was 0.886.

Flavor intensity estimated in the 40 lots of cheese by professional judges showed a linear relationship to soluble tyrosine.

Classical methods for following proteolysis during ripening of cheese depend upon measuring the increase of various forms of soluble nitrogen. Usually, nitrogen in cheese extracts is measured by the Kjeldahl procedure, which is laborious and time-consuming, especially when large numbers of samples are involved.

Colorimetric methods more recently have been developed to estimate extent of proteolysis in ripening cheese. Kosikowski and Dahlberg (8) developed an analytical procedure for measuring tyramine content of cheese colorimetrically. Silverman and Kosikowski (12) modified and shortened this procedure. They believed that total tyrosine liberated in aging cheese was a more sensitive criterion of ripening than the soluble protein content (13).

Mogensen (11) measured colorimetrically the total tryptophane and tyrosine + tryptophane released by hydrolysis in hydrochloric acid filtrates of cheese, to estimate proteolytic decomposition. Correlation between tryptophane and soluble nitrogen was not as high as expected. The discrepancy was attributed to the interference of peptide bonds with the chromogenic properties of these two amino acids and to the fact that tryptophane is easily decomposed by various enzymes.

It seemed that a method of measuring tyrosine and tryptophane that would be independent of peptide bonding might improve the correlation between the

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amounts of these amino acids and the soluble nitrogen content of cheese. The spectrophotometric behavior of these amino acids suggested such an interesting possibility. Their absorption of light in the ultraviolet range is not appreciably affected by peptide linkages. Further, they can be spectrophotometrically determined in admixture, without prior hydrolysis, by the application of Vierordt's theory of analyzing two component systems (16). Holiday (7) and Goodwin and Morton (3) have successfully applied this theory in spectrophotometric determination of tyrosine and tryptophane content in proteins.

There is also the possibility that the soluble nitrogen in a cheese extract might be measured by the optical density of the extract at the wave length of its maximum absorption. This is a common, rapid method for quantitative determination of proteins.

The hypothesis upon which this study is based is that the concentrations of total tyrosine and total tryptophane (free and in peptide linkages) in a sodium citrate–HCl extract of cheese, and the optical density of this extract, are in constant proportion to the soluble nitrogen content of the cheese. The multiplicity of agents involved in cheese ripening, and the fact that tyrosine and tryptophane are themselves decomposed beyond the amino acid stage by bacteria and enzymes, indicate the necessity of careful statistical evaluation of these criteria.

PRELIMINARY EXPERIMENTS

The effect of peptide linking of tyrosine and tryptophane on ultraviolet (U. V.) absorption, and the interference of other amino acid components of casein, were studied by preparing five solutions of known composition and testing their spectrophotometric behavior. These solutions were: *(a)* a simulated casein hydrolysate made by adding pure amino acids to sodium citrate–hydrochloric acid buffer solution at pH 4.5 in the proportions in which they are present in whole casein (9); *(b)* the simulated casein hydrolysate of *(a)* without phenylalanine; *(c)* a sodium citrate–hydrochloric acid buffer solution, pH 4.5, containing only tyrosine and tryptophane in the same proportion and concentration as in *(a)*; *(d)* the simulated casein hydrolysate of *(a)* without tyrosine, tryptophane, and phenylalanine, and *(e)* a solution of pure phenylalanine. The absorption spectra of these five solutions in the range from 235 to 310 mμ are presented in Figure 1. It is significant that the solutions lacking tyrosine and tryptophane showed negligible absorption at wave lengths greater than 260 mμ.

Solutions of nonhydrolyzed whole casein were prepared at pH levels of 1.2, 3.3, 5.57, and 12.2. Absorption spectra of these solutions are shown in Figure 2. The curves of the acid systems are notably similar to those of the casein hydrolysate and the tyrosine-tryptophane system pictured in Figure 1.

The results of these tests with known solutions indicate: that the absorption of the casein systems in the U.V. range of 260 to 305 mμ mainly is due to tyrosine and tryptophane; that the absorbency of these amino acids is not appreciably affected by peptide linkages or by the presence of the other component amino acids, and that the casein hydrolysate can be considered as a two-component system of tyrosine and tryptophane with respect to absorption of U.V. irradiation.

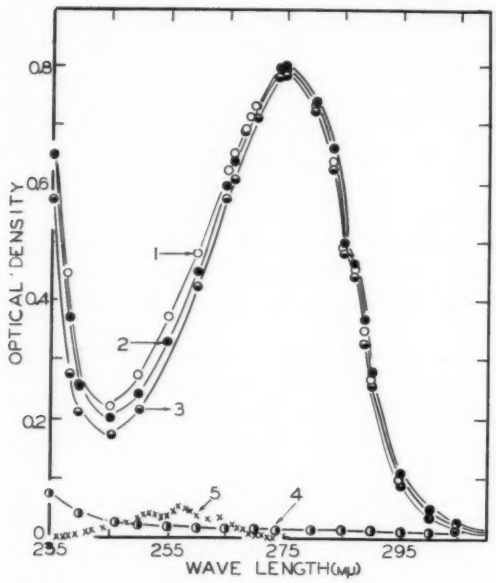

FIG. 1. Absorption spectra of various casein components in sodium citrate–HCl buffer at pH 4.5: – 1 = simulated casein hydrolysate; 2 = simulated casein hydrolysate less phenylalanine; 3 = tyrosine-tryptophane mixture (proportion as in casein); 4 = simulated casein hydrolysate less tyrosine, tryptophane, and phenylalanine, and 5 = pure phenylalanine.

These results obtained with casein systems are in agreement with other spectrophotometric data on proteins found in the literature (1). That the hydrolysis of a protein does not essentially affect its absorption spectrum has been reported in experiments which show that the absorption curves of serum globulin and its tryptic hydrolysate are essentially the same (5).

The effect of the alkaline pH, which shifted the absorption peak of casein shown in Figure 2, is mainly caused by the ionization of the phenolic —OH of tyrosine, the pK value of which is 10–10.1. This effect has been studied by Stentröm and Reinhard (15). Groh *et al.* (4) have reported that the absorption maxima of serum globulins from Blood Group A increased markedly in alkaline solutions. To obtain virtual homogeneity of ionic species, spectrophotometric measurements should be made at a pH less than 8 or more than 12. The pH of 4.5 of the cheese extract used in this study is in a desirable range.

DEVELOPMENT OF ANALYTICAL PROCEDURES FOR CHEESE

Sodium citrate–cheese solution. The procedure of preparing this solution is essentially that of Mogensen (10). Ten grams of cheese, 40 ml. of 0.5 *M* sodium citrate solution, and approximately 80 ml. distilled water are mixed in an Oster mixer at high speed for 7 min. The homogeneous milky solution is quantitatively

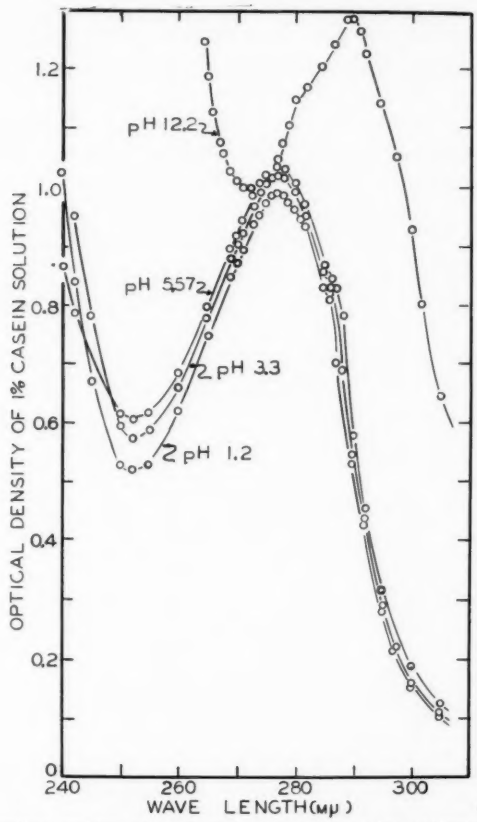

FIG. 2. Absorption spectra of nonhydrolyzed casein at the indicated pH values.

transferred to a 200-ml. volumetric flask, tempered to 20° C., and distilled water is added to the mark with thorough mixing.

Hydrochloric acid filtrate. Ten milliliters of 1.41 *N* hydrochloric acid is added to 100 ml. of the sodium citrate–cheese solution, and the mixture is made up to 125 ml. with distilled water. The pH should be 4.4 ± 0.05. The mixture is then filtered through Whatman No. 42 filter paper. A clear sodium citrate–hydrochloric filtrate is thus obtained which contains the hydrolyzed portion of cheese protein soluble at pH 4.4. This filtrate is referred to as cheese extract, and the nitrogen of the cheese present in this extract is called soluble nitrogen.

Determination of soluble nitrogen. Soluble nitrogen was determined by a modification of the Hiller, Plazin, and Van Slyke semimicro-Kjeldahl procedure (6). Three milliliters of cheese extract was used for this determination.

Spectrophotometric measurements. An aliquot portion, e.g., 25 ml. of the cheese extract, is diluted with the same volume of distilled water; this raises

the pH from 4.4 ± 0.05 to 4.5 ± 0.05. This dilution is made to bring the optical density in the range of from 0.3 to 1.0, in which the Beckman DU spectrophotometer gives accurate and reproducible results. One-centimeter quartz cuvettes are used.

Solvent which is made with 20 ml. of 0.5 *M* sodium citrate solution, 10 ml. of 1.41 *N* hydrochloric acid, and 220 ml. distilled water is used for compensation in the spectrophotometric measurements.

Wave length of maximum absorption of cheese extracts. Forty lots of Cheddar cheese from different factories in Wisconsin and New York were used. They ranged in age from 2 wk. to 30 mo. Sodium citrate–HCl extracts were made from each lot; these extracts were entirely clear and suitable for spectrophotometric studies. Absorption spectra of each lot were prepared over a range of wave lengths from 240 to 310 mμ. The peaks of all absorption spectra were between 274 and 275 mμ.; thus, the maximum absorption of the cheese extract was established at 274.5 mμ. Figure 3 shows a few of these spectra from lots including the full range of age represented in the 40 lots.

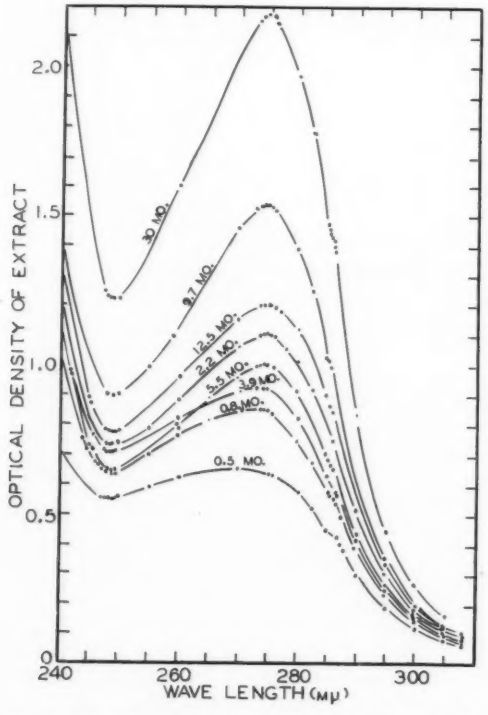

FIG. 3. Absorption spectra of sodium citrate–HCl cheese extracts at pH 4.5 taken from Cheddar cheeses of varying ages. Maxima approximate 274.5 mμ.

Measurement of tyrosine and tryptophane in a mixture, in sodium citrate–HCl buffer (pH 4.5). According to Vierordt's theory, the concentration of each component in a two-component system can be determined by measuring the extinction (E) of the mixture at two wave lengths, and by knowing the molar extinction coefficients of each component at the selected pair of wave lengths. Each component should obey Beer-Lambert's law of absorption.

Molar extinction coefficients (i.e., the ratio of optical density to concentration) for pure tyrosine and tryptophane were determined in sodium citrate–HCl solvent at pH 4.5. The maximum molar coefficient for tryptophane, 5,550, was reached at a wave length of 278.5 mμ; that for tyrosine, 1,420, was observed at 274.5 mμ. Figure 4 illustrates the relationships. Each point is the average of

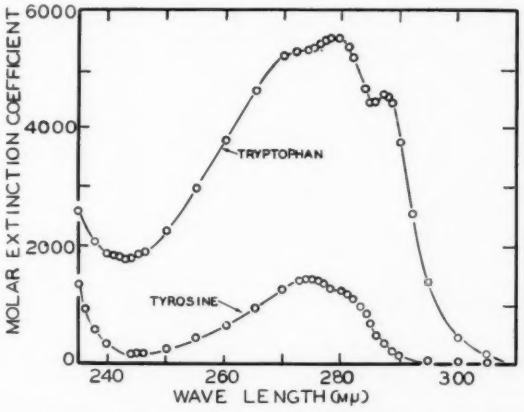

FIG. 4. Absorption spectra of tyrosine and tryptophane in sodium citrate–HCl buffer at pH 4.5. (Each point is the average of six different measurements.)

six determinations, each made with a solution of definite, known concentration. The six concentrations for tyrosine ranged from 0.1 to 0.5 m*M* per liter solution and those for tryptophane from 0.04 to 0.12 m*M* per liter. The molar extinction coefficients, over these ranges of concentration at wave lengths in the range from 260 to 295 mμ, were constant, within the limits of variation expected for such measurements, showing conformity of these two amino acids to the Beer-Lambert law of absorption.

To select the pair of wave lengths at which the two components in mixture could be most accurately determined, a number of known mixtures of tyrosine and tryptophane, varying in concentrations and ratios, were prepared. Optical densities of each mixture were measured at various combinations of wave lengths. Vierordt's formulas (7) were then applied, using the resulting values and the molar extinction coefficients of tyrosine and tryptophane for the specific wave lengths in each instance. The pair of wave lengths, 270 and 290 mμ, gave the most accurate results. Recovery data are shown in Table 1. The error in re-

TABLE 1

Recovery of tyrosine and tryptophane by spectrophotometric measurements from a mixture of the two in a sodium citrate–HCl buffer solution at pH 4.5

Pair of wave lengths used	Tyrosine (*moles per liter*)			Tryptophane (*moles per liter*)			Molar ratio
	Actual	Found	Per cent recovered	Actual	Found	Per cent recovered	
(*mμ*)							
			Mixture I				
270,290	0.667	0.674	101.80	0.667	0.674	101.05	1:1
270,295	0.667	0.724	108.51	0.667	0.661	99.10	1:1
280,295	0.667	0.716	107.43	0.667	0.660	98.95	1:1
280,305	0.667	0.153	22.9	0.667	0.785	117.64	1:1
			Mixture II				
270,290	0.400	0.384	97.50	0.800	0.804	100.52	1:2
270,295	0.400	0.504	126.12	0.800	0.776	97.00	1:2
280,295	0.400	0.499	124.75	0.800	0.776	97.00	1:2
280,305	0.400	0.158	39.45	0.800	0.851	106.36	1:2
			Mixture III				
270,290	1.00	1.008	100.80	0.500	0.508	101.60	2:1
270,295	1.00	0.997	99.65	0.500	0.510	102.00	2:1
280,295	1.00	0.989	98.90	0.500	0.567	113.38	2:1
280,305	1.00	0.509	50.90	0.500	0.616	123.16	2:1
			Mixture IV				
270,290	3.37	3.33	98.78	0.588	0.599	101.96	5.73:1[a]

[a] This is the molar ratio in which tyrosine and tryptophane reportedly exist in whole casein.

covery experiments approximated ± 2% of the actual amino acids present in the mixture.

The equations in their final form are:

$$M_{ty} = (0.95\,E_{270} - 1.31\,E_{290}) \times 10^{-3} \quad (1)$$

$$M_{tr} = (0.307\,E_{290} - 0.020\,E_{270}) \times 10^{-3} \quad (2)$$

where M_{ty} and M_{tr} express moles of tyrosine and tryptophane, respectively, per liter of solution containing the mixture of these two amino acids; and where E_{270} and E_{290} are the optical density values of this solution at 270 and 290 mμ, respectively.

Estimation of soluble tyrosine and soluble tryptophane in cheese extract. Tyrosine and tryptophane present in the cheese extract in the form of free amino acids and in peptide linkages are referred to as soluble. Some of the decomposition products of these two amino acids may contribute to the absorbency and, thus, may be included in these two expressions.

These two products can be determined by applying equations (1) and (2). Measurements required are the optical densities of the 1:1 diluted cheese extract at 270 and 290 mμ. These measurements are substituted in Equations (1) and (2), and the calculated values thus obtained are doubled to give concentrations of soluble tyrosine and soluble tryptophane in moles per liter of undiluted cheese extract. Soluble tyrosine and soluble tryptophane can be expressed as milligrams per 100 g. of cheese by multiplying concentration in millimoles per liter of cheese extract by the factor 453 for tyrosine and 510.5 for tryptophane.

RESULTS AND DISCUSSION

Table 2 shows data on age of cheese, flavor intensity, soluble nitrogen content by the Kjeldahl procedure, optical density of cheese extract at the wave length

TABLE 2

Observations on 40 lots of Cheddar cheese and cheese extracts obtained from them

		Measurements on cheese extract				
		Soluble nitrogen as per cent of		Optical density at 274.5 mμ	Soluble tyrosine	Soluble tryptophane
Age	Flavor intensity	Cheese	Total nitrogen			
(mo.)		—*(%)*—			—*(millimoles per liter)*—	
13.2	Mellow	1.08	29.04	1.126	0.528	0.0850
5.3	Mild	0.75	19.98	0.826	0.294	0.0892
1.3	Mild	0.45	11.91	0.590	0.235	0.0620
3.7	Mild	0.67	17.15	0.870	0.279	0.1020
3.9	Mellow	0.65	13.36	0.830	0.335	0.0906
1.3	Mild	0.46	12.12	0.696	0.156	0.0910
3.7	Mild	0.52	13.96	0.586	0.268	0.0716
2.0	Mild	0.67	16.63	0.860	0.288	0.0965
3.9	Mild	0.65	16.54	0.926	0.350	0.0941
19.1	Mellow	1.34	33.69	1.810	0.550	0.1990
3.1	Mild	0.57	14.91	0.760	0.263	0.0840
17.4	Sharp	1.52	37.44	1.976	0.672	0.2056
1.3	Fresh	0.54	13.88	0.684	0.207	0.0804
6.7	Mellow	0.91	24.38	1.224	0.440	0.1224
6.7	Mild	0.92	24.57	1.280	0.428	0.1354
14.0	Sharp	1.67	43.30	2.200	0.755	0.2250
1.0	Mild	0.58	14.83	0.666	0.166	0.0827
6.8	Mild	0.89	23.53	1.140	0.392	0.1178
6.9	Mellow	0.88	22.97	1.236	0.419	0.1335
0.5	Fresh	0.53	13.58	1.016	0.253	0.1338
14.7	Mellow	1.20	30.54	1.608	0.564	0.1626
0.4	Fresh	0.48	12.13	0.954	0.297	0.1138
9.3	Mellow	1.13	30.00	1.480	0.470	0.1602
2.0	Mild	0.70	18.21	0.985	0.268	0.1195
1.2	Fresh	0.59	15.57	0.868	0.273	0.1018
9.0	Mild	0.76	18.96	1.188	0.277	0.1532
5.5	Mild	0.88	23.19	1.000	0.325	0.1057
2.2	Mild	0.70	17.67	1.096	0.353	0.1217
0.5	Fresh	0.55	14.03	1.078	0.296	0.1062
0.8	Mild	0.55	14.23	0.848	0.288	0.0944
9.7	Mellow	1.22	30.89	1.532	0.612	0.1383
3.9	Mild	0.97	24.18	1.374	0.479	0.1423
14.7	Mellow	1.16	30.38	1.552	0.496	0.1663
0.8	Mild	0.54	14.24	0.842	0.287	0.0939
12	Sharp	1.37	36.21	1.822	0.630	0.1877
5.7	Mild	1.13	29.95	1.550	0.600	0.1461
6.0	Mellow	1.10	28.86	1.558	0.520	0.1666
30.0	Sharp	1.68	43.26	2.174	0.842	0.1956
0.5	Fresh	0.30	8.12	0.636	0.221	0.0731
12.5	Mellow	1.04	26.91	1.194	0.483	0.1266

of maximum absorption, 274.5 mμ, and concentration of soluble tyrosine and soluble tryptophane in cheese extract from 40 lots of Cheddar cheese.

Optical density of cheese extract and soluble nitrogen. The relationship between the optical density of the cheese extract at 274.5 mμ, and soluble nitrogen

measured by the Kjeldahl procedure in the same extract and expressed as per cent of cheese is highly significant. The coefficient of correlation is 0.958. Figure 5 shows this relationship with confidence limits of individual values and population values (14).

Concentration of soluble tyrosine and soluble tryptophane and soluble nitrogen. The concentration of soluble tyrosine and soluble tryptophane in the cheese

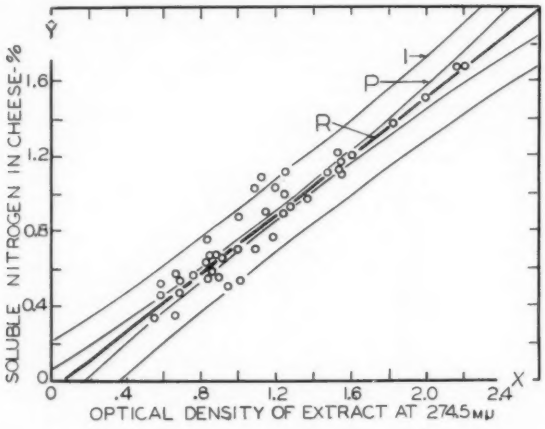

FIG. 5. Relation of per cent of soluble nitrogen in cheese ($\hat{Y}$) to optical density of cheese extract at 274.5 mμ ($E_{274.5}$) shown by regression line (*R*), and 95% confidence limits of individual values (*I*) and population values (*P*). $\hat{Y} = -0.048 + 0.777\ E_{274.5}$; $r = 0.958$; $S_{yx} = 0.102$.

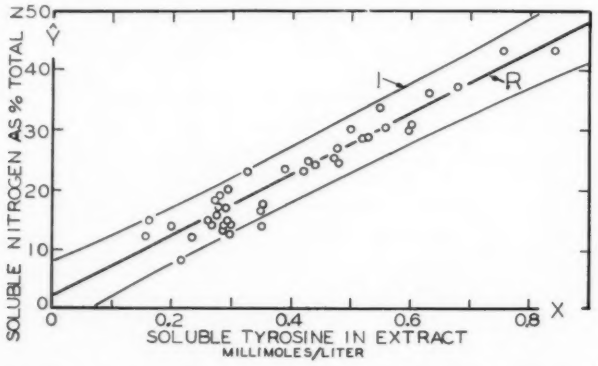

FIG. 6. Relation between soluble nitrogen as per cent of total nitrogen ($\hat{Y}$) and spectrophotometric determination of soluble tyrosine in the cheese extract (*X*) as shown by regression line (*R*), and 95% confidence limits of individual values(*I*). $\hat{Y} = 2.427 + 50.725$; $r = 0.928$; $S_{yx} = 0.101$.

extract are closely related to the percentage of soluble nitrogen of cheese extract as measured by Kjeldahl. These relationships, expressed by coefficients of correlation of 0.964 and 0.886, respectively, are shown in Figures 7 and 8, with 95%

confidence limits for individual and population values of soluble nitrogen at definite values of soluble tyrosine and soluble tryptophane concentrations. Figure 6 differs from Figure 7, in that soluble nitrogen is expressed as per cent of total nitrogen in cheese. Moisture differences in the cheeses are reflected in the differences between the coefficients of correlation shown in Table 3 for each relationship.

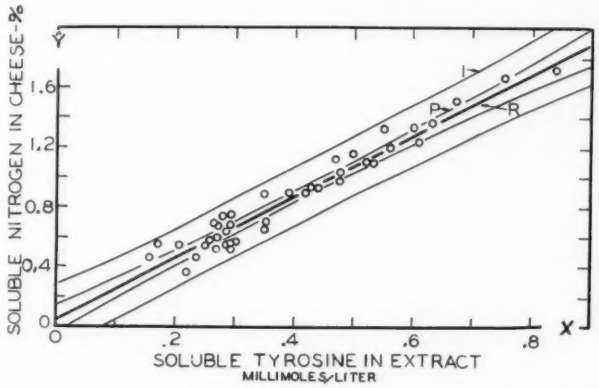

FIG. 7. Relation between per cent of soluble nitrogen in cheese $(\hat{Y})$ and spectrophotometric measurement of tyrosine in cheese extract (X) as shown by regression line (R), and 95% confidence limits of individual (I) and population (P) values. $\hat{Y} = 0.055 + 2.032\,X$; $r = 0.964$; $S_{yx} = 0.098$.

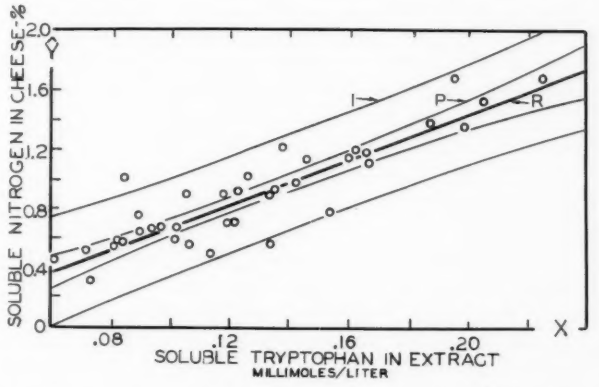

FIG. 8. Relation between per cent of soluble nitrogen in cheese $(\hat{Y})$ and spectrophotometric measurement of soluble tryptophane in cheese extract (X) as shown by regression line (R) and 95% confidence limits of individual (I) and population (P) values. $\hat{Y} = -0.088 + 7.600\,X$; $r = 0.886$; $S_{yx} = 0.165$.

Four least-square regression equations have been developed from the data for the above relationships. They are presented in Table 3 with the standard error of estimate (S_{yx}) and correlation coefficient (r).

TABLE 3

Relation of optical density at 274.5, concentration of soluble tyrosine, and concentration of soluble tryptophane in cheese extract to the soluble nitrogen content of cheese

No.	Regression equations	Correlation coefficient (r)	Standard error of estimate (S_{ys})
(1)	$N_S = -0.048 + 0.777\ E_{274.5}$	0.958	0.102
(2)	$N_S = 0.055 + 2.032\ M_{ty} \times 10^3$	0.964	0.098
(3)	$N_S\ \%\ N_T = 2.427 + 50.725\ M_{ty} \times 10^3$	0.928	0.101
(4)	$N_S = -0.088 + 0.760\ M_{tr} \times 10^4$	0.886	0.165

N_S = Soluble nitrogen per cent cheese.
$N_S\ \%\ N_T$ = Soluble nitrogen per cent of total nitrogen.
$E_{274.5}$ = Optical density of cheese extract at 274.5 mμ.
M_{ty} = Moles soluble tyrosine per 1,000 ml. cheese extract.
M_{tr} = Moles soluble tryptophane per 1,000 ml. cheese extract.

Comparison of the standard errors of estimate for the equations shows that Equation 2 has the lowest standard error of estimate and Equation 4 the highest. These results would indicate that the quantity of soluble tyrosine (M_{ty}) estimates soluble nitrogen in the cheese (Ns) more accurately than soluble tryptophane (M_{tr}), and that optical density at 274.5 mμ (E 274.5) of cheese extract is a satisfactory measure of soluble nitrogen. The correlation coefficients also indicate that M_{ty} and N_s are more closely related than either M_{tr} and N_s or E 274.5 and N_s. When soluble nitrogen is expressed as per cent of total nitrogen, the correlation coefficient is considerably reduced, although there is only a slight change in the standard error.

Soluble tyrosine, according to the data shown in Table 3, would seem to be the best estimate of cheese ripening, insofar as ripening is indicated by increase of soluble nitrogen in the cheese extract. The lower correlation between soluble tryptophane and soluble nitrogen may be attributed to the decomposition of tryptophane by enzymatic processes, so that it no longer absorbs ultraviolet light.

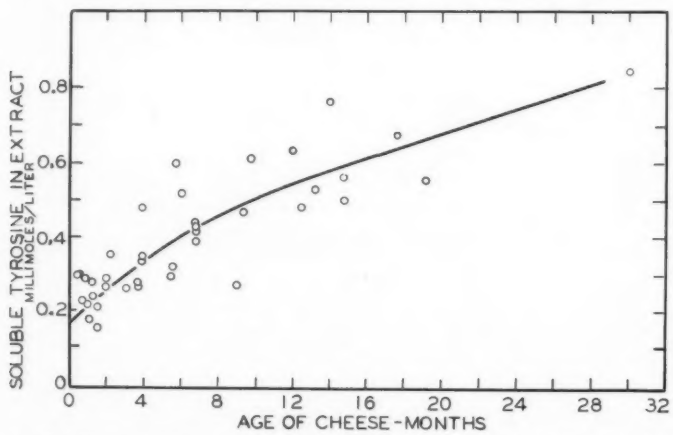

FIG. 9. Relation between spectrophotometric determination of soluble tyrosine in cheese extract and the age of cheeses made in Wisconsin and New York factories.

Age of cheese and absorbency of cheese extract. Figure 3 shows the ultraviolet absorbency of extracts from eight lots of cheese of different ages and different factories of origin. There is a tendency for increasing optical density of the cheese extract to be associated with advancing age in the cheese from which the extracts were obtained, but there are exceptions. Curves in Figure 3 for the lots aged 0.5, 3.9, 5.5, 12.5, and 30 mo. were obtained from cheese produced at the University of Wisconsin; the age and optical density relationship is obvious. But the other curves of Figure 3 were derived from lots of cheese from different factories, and they do not fall in order of age with the University lots. Such variations in the broad relationship must be expected because of the wide differences possible in methods of manufacture, including heat treatments of milk, as well as in amounts and types of biological agents responsible for rate of ripening and nature of protein decomposition.

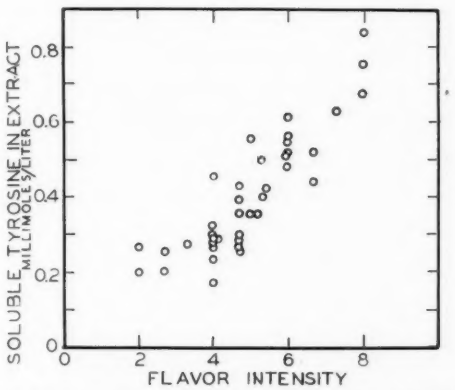

FIG. 10. Relation between spectrophotometric measurements of soluble tyrosine in cheese extract and flavor intensity of cheese evaluated by professional judges.

Age of cheese and soluble tyrosine in cheese extract. Figure 9 shows the trend of the relation between the age of cheese and the soluble tyrosine in the cheese extract. Soluble tyrosine tends to increase more rapidly in the early stages of ripening. The deviations of observations from the curve drawn by inspection to fit best the data are to be expected in view of the wide differences in origins of the lots of cheese.

Flavor intensity and soluble tyrosine in cheese extract. Three professional judges classified the intensity of cheese flavor of the 40 lots of cheese as either fresh, mild, mellow, or sharp. These four flavor classes were given arbitrary values of 2, 4, 6, and 8, respectively, to calculate the coefficient of correlation ($r = 0.805$) between flavor as an indication of age and soluble tyrosine.

The results of these observations and measurements are plotted in Figure 10. The trend of the relationship is of interest, especially in view of the complicated bacterial and enzymatic activities responsible for the magnitude of the obser-

vations. Dahlberg and Kosikowski (2) observed a direct, semilogarithmic relationship between tyramine content and intensity of flavor of Cheddar cheese, even though tyramine was not the Cheddar flavor compound.

CONCLUSION

The spectrophotometric method is a rapid procedure for estimating the extent of cheese ripening. It is faster and has an accuracy approximately equal to that of measurements of soluble nitrogen in an extract of the cheese by the Kjeldahl procedure.

ACKNOWLEDGMENTS

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EFFECT OF SOIL FERTILITY UPON THE YIELD AND NUTRITIVE VALUE OF FORAGES. A REVIEW [1, 2]

GEORGE M. WARD

Department of Dairy Husbandry, Kansas State College, Manhattan

SUMMARY

The effects of fertilization of forage crops vary with soil types, relative levels of fertility within soil type, ratios of available nutrients, crops, and climatic conditions. Increased forage yields result from application of the plant-growth nutrient which is most limiting in the soil.

The chemical composition of forages may be altered by fertilization. Application of a nutrient in quantities greater than those required for maximum yield response usually results in luxury consumption of the nutrient.

The nutrients whose level in plants may be increased by large applications include nitrogen, phosphorus, potassium, calcium, sulfur, and cobalt. The nitrogen and phosphorus levels in leguminous forage usually were not influenced by heavy applications of these nutrients.

Biological assays of forages produced with different fertilizer treatments have yielded varying results. Several studies with sheep and rabbits have indicated that applications of phosphorus fertilizer increase the biological value of forages when these forages are fed alone or in highly simplified rations. Application of limestone to the soil has been shown to exert a favorable effect on the biological value of forage. Forages grown on light soils have lower biological value for guinea pigs than do forages grown on heavy soils. Other, more numerous reports indicate no difference in biological value of forage due to level of soil fertility.

BASIC CONCEPTS

The relationship between relative levels of soil fertility and the nutritive value of the crops grown on the soil has been the subject of much study and some conjecture. Various factors, grouped in climate, soil, and plant categories, modify the influence of soil fertility level on the yield and chemical composition of the crop grown.

It has been firmly established that at least 12 elements must be available if normal plant growth is to be obtained (118). Some of these are quantitatively more important than others. Nitrogen, phosphorus, and sulfur are directly involved as protein and nucleic acid constituents. Since the content of the elements is fixed, protein synthesis and content are directly linked to the supply of all three. On the other extreme, zinc, molybdenum, copper and, possibly, chlorine have catalytic roles in metal-protein complexes. The requirements for these elements are not linked directly to any major organic constituents of plants. In less extreme position are the cations—potassium, calcium, sodium, and magnesium—which have multiple roles and among which there is a considerable degree of interchangeability as well as competition. The minimum requirement

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[2] Presented at the joint meeting of the American Grassland Council and the American Dairy Science Association at North Carolina State College, Raleigh, June, 1958.

of this group as a whole is related to the amount of plant growth and differs greatly among species.

Inorganic nutrient absorption in the plant is not orderly in the sense of a manufacturing process. It is simply an exchange process, somewhat selective but not regulated by the needs of the plant. The rate of nutrient uptake is more closely related to the supply in the soil than to the requirement of the plant.

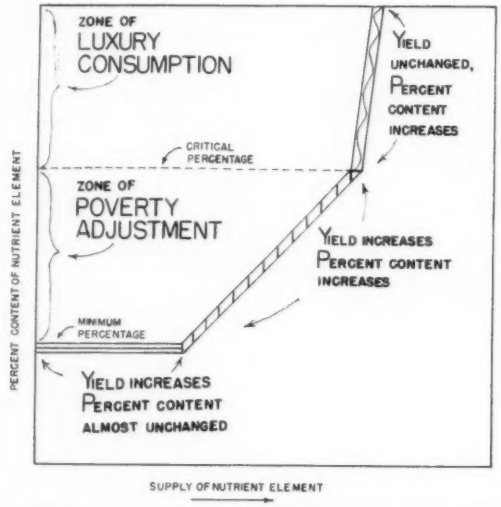

FIG. 1. Relationship among (1) supply of nutrient in the soil, (2) concentration of that nutrient in the crop, and (3) the yield of crop (Norman, 118).

Figure 1 describes the relationship among supply of the nutrient element in the soil, the yield of the crop, and the concentration of the nutrient in the crop. A certain minimum concentration of each element is necessary to obtain any growth of the plant. Increased amounts of the nutrient in the soil yield more crop having the same concentration in the plant to a point. Beyond this point, increase in supply of the nutrient element results in increases both in yield of crop and concentration of nutrient—the zone of poverty adjustment. Beyond this point, luxury consumption is in effect and only increased concentration of the element is noted.

This situation would be relatively simple if only one nutrient were involved. It is extremely complex when many nutrients are considered. Fertilization may change the status of one nutrient from poverty adjustment to luxury consumption, while reversing this situation for some other element due to increased yield of the crop.

Animals require cobalt and iodine in addition to all the elements needed by plants, except boron. Only by rare happenstance would a plant have all the nutrients in the proper proportions for efficient utilization of each by an animal.

The need for mixed rations for efficient animal production has been recorded repeatedly.

Various aspects of this subject have been previously (14, 15, 16, 33, 54, 56, 81, 107, 131, 155, 164).

YIELD

The effects of the various rates of fertilization and the different proportions of plant nutrients supplied vary with a number of factors. Soil types and the relative level of fertility within soil type exert considerable influence on the crop-yield response from fertilizer application. The ratio of the available nutrients within the soil is a factor which modifies the results expected from specific fertilizers. The addition of the nutrient which is limiting plant growth results in considerable yield increase, whereas the addition of a nutrient which is already in sufficient supply in the soil produces little or no increase. The response to fertilizers varies with the species or mixture of species being studied. Results of soil fertility experimentation are dependent, to a considerable extent, on the rainfall, sunshine, wind, heat, and humidity relative to the requirements and adaptability of the species under study.

Nitrogen. The effect of nitrogenous fertilizers on the yield of various forage crops has been studied extensively. Nitrogen application has resulted in increased yield of grasses in many experiments (13, 22, 36, 39, 42, 43, 48, 63, 66, 67, 72, 76, 77, 79, 86, 95, 97, 100, 106, 112, 116, 117, 127-130, 134, 137, 140, 149, 150, 151, 160, 165, 170) but has produced little or no effect in others (83, 98). Some reports have indicated that this nutrient depresses the yield of legumes in mixed forage (22, 36, 116, 117), although others indicated little or no change (43, 48, 98, 111).

The level of application of nitrogen fertilizers and the relative balance of nutrients in the soil are factors affecting the results obtained. Burton *et al.* (39) increased early growth of coastal Bermuda grass phenomenally with heavy applications of nitrogen (Table 1). Two hundred pounds of nitrogen appeared to be the most effective level of application in this case. Carter (42) reported an increase in bromegrass hay yields from one ton per acre on the check plot to three tons per acre with the application of 133 lb. of nitrogen per acre.

The response to nitrogen fertilization depends to considerable extent on the

TABLE 1

Effect of nitrogen fertilization on yield and protein content of 4-wk. clippings of Coastal Bermuda grass (Burton *et al.*, 39)

Applied N	Yield *(4 wk.)*	Protein
(lb/A)	*(lb. DM/A)*	*(%)*
0	1,154	12.3
50	2,199	12.7
100	3,053	14.3
200	3,569	14.7
400	3,762	18.1
900	4,061	20.0
1,500	2,342	20.8

availability of other nutrients in the soil. Fisher *et al.* (63) showed that one Texas pasture responded adequately to nitrogen only with concurrent application of phosphates (Table 2). Other workers (83, 130, 150) have reported the

TABLE 2

Effect of fertilizers on the yield of pasture forage in Texas (Fisher *et al.*, 63)

N	P_2O_5	K_2O	Yield
(lb/A)			*(T/A)*
0	0	0	2.9
0	0	150	2.6
0	150	0	3.5
0	150	150	3.4
450	0	0	4.9
450	0	150	4.6
450	150	0	7.8
450	150	150	8.3

need for phosphate to obtain maximum response to nitrogen fertilization. Holmes and MacLusky (79) found that heavy applications of potash were necessary to obtain large responses to nitrogen fertilization. Holmes (77) reported that continued heavy applications of nitrogenous fertilizers resulted in declining yields unless phosphate and potash were applied, indicating that the high-level nitrogen fertilization depleted the phsophate and potash contents of the soil.

Phosphorus. The response to phosphorous fertilzation depends to a large degree upon the relative proportion of legumes in the forage and on the level of available phosphorus in the soil. Increases in yields of forage with application of phosphates have been found in many cases (4, 29, 38, 40, 45, 63, 73, 80, 86, 91, 94, 99, 102, 104, 105, 113, 115, 121, 123, 139, 142, 148, 161, 171, 174, 175). Some cases of insignificant change in yield after application of phosphates have been reported (20, 24, 37). Welch *et al.* (168) found that yields of Ladino clover were significantly correlated ($P = < .01$) with soil test phosphorus values. Pittman (123) found that yield of alfalfa and the percentage of phosphorus in the forage were closely correlated on responsive soils, but not correlated significantly on unresponsive soils.

Potash. Winters (173) reported that the yield of alfalfa was not increased by potash fertilization if the soil contained more than 160 lb. of exchangeable potash per acre. Chandler *et al.* (44) found that fertilization of alfalfa with potash was profitable if the exchangeable potash content of the soil was less than 80 lb. per acre, or if the potassium content of the forage was less than 1.25% of the dry matter at the early-bloom stage of maturity. Small and frequent applications of potash, where necessary, were found to be desirable and economical means of maintaining yields (32, 44). Large, single applications resulted in luxury consumption of the nutrient and faster removal of the potash from the soil. Increases in forage yields were obtained with potash applications in a number of cases (25, 63, 79, 113, 142, 143, 147, 148). Yield of forage was not influenced in some cases of potash fertilization, presumably due to the presence of sufficient potash in the soil (86, 101, 132).

Mixed fertilizers. In practically every study reported, the application of mixed fertilizer resulted in increased yields of forage (1, 2, 6, 25, 47, 55, 64, 68, 70, 120, 132, 153).

Calcium. The effect of the application of calcium on the yield of forage crops depends on the pH of the soil, the amount of calcium applied, the nature of the calcium compound, and the established flora—legumes responding more favorably to more nearly neutral soil. Increased forage yields due to liming were found in most instances (25, 29, 85, 139, 152, 174, 175) (Table 3), whereas

TABLE 3

Effect of limestone on the yields of alfalfa and lespedeza (Woodhouse, 175)

Limestone	Alfalfa	Lespedeza
	(lb/A)	
0	1,025	1,987
1,000	1,832	2,541
3,000	3,382	2,700
L.S.D. $p < .05$	528	244

insignificant change in the yield has been recorded on predominantly grass swards (26, 132). Raising the pH of soil by liming promotes the establishment of legumes, particularly alfalfa and the clovers (9, 13, 34, 156), although phosphate application may be necessary to realize this effect in some cases (28).

Boron. Legumes seem to be particularly sensitive to deficiency of boron in the soil. Excessive liming tends to make soil boron unavailable to plants. Borax applications have increased yields of legumes phenomenally in some cases (113, 133). Other reports (34, 35, 44) indicate that yield response from borax application may not be obtained, even though signs of boron deficiency are apparent on the plants. Dawson and Gustafson (52) concluded that boron deficiency occurs when the boron content is less than 20 p.p.m. dry matter in alfalfa or less than 0.35 p.p.m. air-dry soil.

Sulfur. Reports from California (7) and New Zealand (162) claim that sulfur is sometimes a limiting nutrient. Since gypsum was the sulfur carrier used in both cases, calcium possibly could have been responsible for the increased forage yields (Table 4).

TABLE 4

Effect of gypsum on the yield and calcium and sulfur content of pasture forage in New Zealand (Walker *et al.*, 162)

Gypsum	DM Yield	Ca	S
(lb/A)		*(%)*	
0	853	1.78	0.17
25	1,116	1.87	0.29
50	1,839	2.01	0.41
100	2,086	2.15	0.30
200	2,302	1.92	0.32
L.S.D. $p < .05$	454		

CHEMICAL COMPOSITION

Variation in the level of fertility and the ratio of nutrients of the soil on which forage crops are grown may cause variation in the chemical composition of the crop in any or all of several ways. The bontanical composition of mixed herbage may be altered. The leaf/stem ratio may be changed. One nutrient may be so plentiful that luxury consumption occurs, resulting in the forage containing an abnormally large proportion of that nutrient. The various species react independently to differences in soil fertility; thus, the results of variations in soil fertility vary with the species and proportions of species in the stand.

Nitrogen. Moderate-to-heavy applications of nitrogenous fertilizers to hay and pasture crops have resulted in increased content of nitrogen in the forage in many cases (18, 39, 41, 42, 43, 53, 59, 60, 61, 67, 71, 74, 76-79, 97, 111, 112, 116, 117, 127, 128, 129, 134, 159, 160, 161, 165, 169, 170) (Table 5). Nitrogen content of

TABLE 5

Effect of nitrogen fertilization on dry matter and protein yield of Orchard grass (Ramage *et al.*, 129)

Applied N	Yield	Protein	
(lb/A)	*(lb. DM/A)*	*(%)*	*(lb/A)*
50	6,400	12.2	516
100	6,850	13.6	779
200	7,950	15.8	1,098
400	9,150	20.1	1,794

legumes in the forage usually was not affected by the fertilization (111, 159, 172) and the nitrogen content of legume-grass mixtures was not influenced in some cases (65, 95, 98, 100, 140, 149), usually due to compensatory decrease in legume population (22, 48, 176). Heavy applications of nitrogenous fertilizers ten to 20 days before cutting hay increased the nitrogen content of the first crop but not that of the aftermath (96, 144, 145). Another report (111) also indicated that the nitrogen content of aftermath usually was not affected. One case of heavy rainfall after nitrogen fertilization resulted in insignificant change of the nitrogen content of the forage produced (74). Observations made on forages in which the nitrogen content had been increased by fertilization have included increased content of nonprotein nitrogen (159), although most of the extra nitrogen was in a complex precipitable by copper (96), decreased content of crude fiber (60), decreased percentage dry matter at immature stages (159), and earlier growth (65). Increased carotene content of forage fertilized with nitrogen has been reported (82, 151).

Phosphorus. Fertilization of various forage crops with phosphates has resulted in increased phosphorus content of the forage in many instances (2, 4, 19, 20, 21, 31, 37, 38, 40, 45, 58, 60, 69, 72, 73, 74, 80, 86, 91, 94, 102, 104, 105, 115, 123, 130, 137, 139, 150, 158, 159, 167, 169) (Table 6). Phosphorus content of phosphate-fertilized forage was not always altered (24, 114). Practically all of the extra phosphorus in the forage due to fertilization is present in inorganic

TABLE 6

Effect of nitrogen and phosphorus applications on yield, protein, and phosphorus of native grassland forage in South Africa (Theron, 150)

Treatment	Yield	Protein	P_2O_5
	(T/A)	——————(%)——————	
0	0.5	6.0	.25
P[a]	0.6	7.9	.58
2P	0.7	6.7	.50
N[b]	1.6	8.1	.16
NP	2.2	6.9	.36
N2P	2.3	8.0	.35
2N	2.1	9.5	.18
2NP	3.5	9.3	.30

[a] P = 60 lb. P_2O_5/A.
[b] N = 100 lb. N/A.

form (102). Increased levels of phosphorus in the cell sap have been reported as a result of phosphate application (8). Larson *et al.* (94) reported that stunted alfalfa produced 0.33 ton per acre and contained 0.14% phosphorus, as compared to normal alfalfa, which produced 1.38 tons per acre and contained 0.17% phosphorus. These differences occurred in samples taken as close as 4 ft. from each other. A highly significant correlation coefficient, .56, between the phosphorus content of alfalfa and the water-soluble phosphate level in the soil on which it was grown, has been reported (119). The critical level for phosphorus in petioles of Ladino clover has been reported to be 600 p.p.m. acetic acid soluble phosphate phosphorus (154). Coefficients of correlation between yields of Ladino clover and soil test phosphorus values obtained by various methods ranged from .72 to .92 (168). Increased protein content of phosphate-fertilized forage has been reported (31, 102, 104, 123, 137), probably due, in some measure, to changes in botanical composition of the forage (30, 60, 73, 132, 146, 147), although phosphate fertilization did not increase the protein content of alfalfa (102, 124). Decreased protein in timothy hay has been reported to be a result of this treatment (102).

Phosphate application has resulted in increased content of calcium in the forage (31, 104, 105, 139, 159) and decreased dry matter content of the fresh forage (159). Concurrent nitrogen and phosphate fertilization (4) and heavy rainfall after phosphate application (74) both have resulted in decreased content of phosphorus in the forage.

Potassium. Potash fertilization has resulted in increased potassium content of forage produced in many cases (2, 8, 22, 35, 62, 72, 73, 92, 142, 143, 156, 157, 158, 161). One report (108) indicated that the potassium content of forage produced on light soils is lower than that in forage from heavy soils. Heavy applications of potash have resulted in decreased levels of calcium (35, 92), magnesium (161), and sodium (161) in the forages. This is due to the effect of cation constancy (12, 35, 84) in which the total milliequivalents of cations in forage remain about the same, whereas the ratios among cations vary. An inverse relationship exists between the contents of calcium and potassium in the forages (1). The critical level for potassium in the petioles of Ladino clover is

TABLE 7

Effect of liming on the calcium and phosphorus contents of alfalfa (Power *et al.*, 124)

pH	Ca	P
	(%)	
4.7	1.62	0.49
5.8	2.19	0.29
6.3	2.36	0.34
6.8	2.57	0.35

approximately 0.8% (154). Potash fertilization also has resulted in decreased per cent dry matter (159), increased nitrogen content (6, 159), increased nonprotein nitrogen content (159), and increased ash content of the forage (124, 159).

Calcium. Increased calcium content of forage usually follows lime applications to the soil (9, 26, 109, 122, 124, 152, 156, 169, 174) (Table 7), but not always (130). Other changes in the composition of forage reported as results of liming are increased proportion of legumes in the mixtures (13, 156), decreased content of phosphorus (124, 138), decreased level of iron (26, 124), decreased level of manganese (26), and increased level of protein (29, 124). More calcium was found in forage grown on light soils than in that grown on heavy soils (108). Liming decreased plant uptake of cobalt, manganese, and zinc (126), and increased molybdenum uptake (171).

Sulfur. The sulfur content of forages was more than doubled due to applications of gypsum (7, 162). Practically all of the forage sulfur was in organic form (162).

Minor elements. Silicon, iron, and aluminum were found in greater concentration in forage from unfertilized soil than from fertilized soil (31). Applications of iron to soil resulted in yield response without increased content of the element in the forage (5). Cobalt fertilization brought about increased levels of cobalt, but also caused the uptake of dangerous quantities of molybdenum in the forage (110). Price *et al.* (126) found that the levels of cobalt, copper, manganese, and zinc in forages varied among soils and years and with fertilization.

Beeson *et al.* (17) grew several forage species under uniform greenhouse conditions, to compare the phosphorus, cobalt, manganese, and copper contents of the species. These investigators found that Kentucky bluegrass contained the greatest concentration of all the elements determined except manganese. Timothy contained the lowest quantities of all minerals studied. Dallis grass, orchard grass, Johnson grass, and red top were intermediate.

BIOLOGICAL VALUE

Guinea pigs, rabbits, sheep, beef cattle, and dairy cattle have been used in an attempt to assay biological values of crops resulting from different fertilizer treatments. A variety of biological measures has been used, including weight gains, carrying capacity, milk and butterfat production, animal health, hay consumption, and composition of tissues.

The effect of added phosphate on the productivity of a Bermuda grass–legume mixture was measured as weight gain of beef cattle by Sandal and Garey (135) (Table 8). Blaser *et al.* (23) reported twice as much weight gain of beef cattle

TABLE 8

Effect of phosphate fertilization of a Bermuda grass–legume mixture on beef cattle growth (Sandal and Garey, 135)

Available P_2O_5	Beef yield
(lb/A)	
11	61
40	275
166	382

on pasture grass fertilized with nitrogen, phosphorus, and potassium as on the unfertilized grass. Daily gains were larger and feed efficiency was greater on the fertilized grass. These workers noted that increases of potassium, calcium, phosphorus, and protein in the forage were associated with the greater weight gains of the cattle. Sandal *et al.* (136) increased beef cattle weight gains to from four to seven times those on unfertilized Bermuda grass mixtures by applying 40–120 lb. of available phosphoric anhydride (P_2O_5) per acre, in the form of superphosphate. Addition of potash and nitrogen was of no benefit in that study. Hall *et al.* (72) found that the weight gains of cattle per acre of South African range were directly proportional to the amount of nitrogen applied.

Pratt and Holdaway (125) reported that fertilization of pastures increased the cow-days per acre, almost doubled milk production per acre, and considerably increased TDN per acre. Eheart and Pratt (57) conducted a 10-yr. pasture fertilization experiment. These investigators concluded that the greatest value of fertilization of pasture was the increase in yield, and that unfertilized bluegrass pasture met the requirements for milk production as well as fertilized pasture, provided enough herbage was available to supply additional energy required to gather herbage from the poorer sod. Their results indicated greater digestibility of protein from fertilized pasture, but no significant differences in 4% FCM per cow between treatments. Lush and Fletcher (101) found that application of nitrogen was most effective in increasing butterfat production per acre of pasture. König (93), using cows, determined the relative consumption of hay fertilized with phosphorus alone and with nitrogen and/or potassium. Hay fertilized with nitrogen, potassium, and high-level phosphorus was consumed in greatest quantities, followed by that fertilized with nitrogen, potassium, and phosphorus, and followed by that which received no potassium fertilization. Body weight increases of the cows were in the order of the hay consumption, with the nitrogen and phosphorus-fertilized hay resulting in weight loss. Potassium fertilization promoted clovers in the forage, thus increasing the protein content of the hay.

Albrecht and associates (3, 75, 141) attempted to correlate performance of rabbits and sheep with the fertility of the soil on which their feed was grown.

Phenomenal differences were obtained among treatments. Lespedeza hay fertilized with phosphorus produced 20–26% greater yield than the unfertilized lespedeza. Lamb gains were 60–80% greater in favor of the fertilized hay. Results with rabbits were in the same order. Timothy hays, unfertilized, and fertilized with phosphorus, limestone, or nitrogen were assayed with lambs fed a constant grain ration. The hay fertilized with nitrogen yielded the best results. Results of one experiment (141) prompted these authors to conclude that maximum yield was not necessarily associated with maximum nutritive value. Beeson (16) criticized these studies because too few animals were used, botanical composition of the crops was not controlled, and feed wastage was exorbitant.

Webb *et al.* (166) compared forage from unfertilized southern Illinois soil with that produced after applications of phosphate and lime for sheep. The major result of the fertilization was more animal product per acre. No significant difference was noted in bone ash, serum phosphate, or reproductive performance of the sheep as the result of the soil treatment. Matrone *et al.* (103) found significant difference between weight gains of lambs fed cerelose and soybean hay grown with and without phosphate fertilization. When the cerelose was replaced by soybeans, the fertilized hay showed no advantage. The protein in the fertilized hay was significantly more digestible, but this was probably related to the quantity consumed, rather than to the quality of the protein. Serum phosphate levels were higher in animals fed the fertilized hay and cerelose than in those fed the unfertilized hay and cerelose. Differences in analysis—feed analysis, calcium, phosphorus—between the two hays were not significant.

Further work by Matrone *et al.* (104) indicated that phosphate fertilization produced from two to three times as much soybean hay as no fertilization, and that the fertilized hay was of greater biological value for sheep and rabbits. The difference was masked when the soybean hay was supplemented with soybeans or corn, but not when cerelose was the supplement. Lambs fed fertilized hay and cerelose gained 17.7 lb., as compared to 12.1 lb. for lambs on the control ration. Addition of dicalcium phosphate to the ration corrected the weight gain, but did not normalize the serum inorganic phosphorus levels. The authors concluded that the lower phosphorus content of the unfertilized soybean hay was the cause of the poor results with lambs. Rabbits fed the fertilized soybean hays as the major portion of their ration gained significantly more weight, had higher levels of serum inorganic phosphorus, required greater pressure to break the femur, and had higher specific gravity of the femur (105). Addition of

TABLE 9

Effect of feeding soybean hays grown with and without added phosphate to lambs (Matrone *et al.*, 104)

Treatment	Feed intake	Weight gain
	(lb/day)	
+ P	1.47	.109
− P	1.23	−.026

0.75% phosphorus to the diet corrected the deficiency encountered with the unsupplemented, unfertilized ration.

Bonnet *et al.* (27) compared the effects of unlimed and limed grass on the chemical composition of goats' blood. No significant difference was found between rations in blood analyses, growth, and animal health.

Crampton (50) fed rabbits forage from unfertilized and phosphorus and potassium fertilized plots. The forage was mixed grass and clover, having 4% clover from the unfertilized area and 15% from the fertilized area. The rations contained the forage, oat hulls, amino acids, and salt. Both rations were adjusted to the same protein content. The ration containing herbage from the fertilized area produced considerably more gain. Casein and cystine supplementation eliminated the difference between rations. Another report (51) corroborated the first and considered the possibility of difference between the protein complexes of the two herbages. Fertilization produced more clover in the forage.

Kendall *et al.* (89) reported that application of limestone to plots already fertilized with phosphorus and potassium yielded forage of greater nutritive value for rabbits than that from unlimed plots. The forage from the limed plots was more palatable, the rabbits produced larger litters, and the weights of the young were greater.

Wedin *et al.* (167) used guinea pigs to assay the nutritive value of forages from different soil types. These workers concluded that the nutritive value of the forages for guinea pigs was closely correlated with the productivity ratings of the soil types. Highly significant decrease in nutritive value was found between forages grown on silt loam and sand. Response in nutritive value due to fertilization varied. The most response was encountered on soil of inherently low fertility.

A 10-yr. Michigan study (163) compared rations composed of hay and grain grown on fertilized and unfertilized soil of the same type. Four successive generations of dairy cattle were utilized on this experiment. The hay from the fertilized soil contained significantly more ash, calcium, phosphorus, potassium, and copper, and significantly less manganese, than that from the unfertilized soil. The grain mixture from the fertilized area contained significantly more phosphorus. Significant difference was not noted between treatments with regard to reproductive efficiency, health, heifer growth, and blood picture. Feed intake varied from year to year and between treatments. Milk production tended to vary with, and in the same direction as, feed intake and the difference between treatments was insignificant when adjusted for feed intake. The relative coarseness of the hay was the most probable influence on feed intake. The hay from the fertilized area was considerably coarser and, consequently, less palatable.

Keener *et al.* (87, 88) have reported some results of feeding timothy, bromegrass, and Ladino clover hays grown on heavily fertilized soil. The crops were low in iron, cobalt, copper, magnesium, and manganese, but normal in calcium and phosphorus. Cattle fed these hays grew poorly, had rough hair coat, anemia, sagging spinal column behind shoulders, ataxia of hind legs (on timothy-fed

calves only), loss of tips of ears, and broken bones. After freshening, cows lost weight rapidly, produced poorly, had depraved appetites, and were nervous. Calves had goiters which were not explainable by the iodine content of the feed. Supplementation of the timothy hay with iron, copper, and iodine did not stimulate calf growth. Rabbits fed the timothy hay supplemented with 5% alfalfa were normal. Neither supplement of the equivalent amount of alfalfa ash nor iodine prevented the symptoms. These workers concluded that the timothy hay was deficient in iodine and some organic constituent(s).

Knox *et al.* (90), in an attempt to ascertain the cause for poor performance of dairy cattle in southeastern Kansas, compared feeds grown in the area, with and without bonemeal supplement, with feeds grown outside the area. Milk production was satisfactory and did not significantly differ among treatments. Underfeeding was suggested as the probable cause of the difficulties encountered with dairy cattle in the area.

Bartlett *et al.* (10) reported that application of 2,500 lb. of magnesite per acre prevented the occurrence of grass tetany in the cows which grazed the treated area. The magnesium intake of the cows was approximately doubled on the treated area, as compared to areas treated with nitrogen, potassium, or not treated.

Chester *et al.* (46) found that top dressing copper-deficient pasture with 14 lb. copper sulfate per acre at 6-mo. intervals was necessary to maintain adequate copper status in grazing cattle.

Cooper and Woodle (49) reported that ratios of nitrogen to phosphorus of more than eleven to one in forage were conducive to bloat. These authors suggested heavy application of phosphate fertilizers as a bloat preventive. Preliminary results of a Kansas study (11) indicate that applications of 400 lb. of available phosphoric anhydride per acre did not prevent bloat with alfalfa. The soil tested in excess of 200 lb. available phosphorus per acre before the application of superphosphate.

DISCUSSION AND CONCLUSIONS

Plants require optimal levels of all the required nutrients for maximum growth commensurate with the prevailing climatic conditions. Deficiency of any one of the required plant nutrients results in less than maximum crop yield. Concurrently, the level of that nutrient in the forage produced is the minimum at which that plant grows. The other nutrients available in more plentiful amounts are present in relatively greater concentrations in the forage.

Additions of one fertilizer nutrient may increase the supply of that nutrient sufficiently to cause its luxury consumption, while another nutrient becomes the limiting factor in crop yield. The relative balance of nutrients for plant growth influences the chemical composition of the forage. Balanced nutrient supply, whether at low or high level, usually results in forage of approximately the same mineral nutrient composition, varying only in yield. Imbalance of plant nutrients results in luxury consumption of the nutrient in abundant supply, with concurrent high level of that nutrient in the crop.

The most serious ramification of low soil fertility is the mediocre-to-poor management with which it is often associated. Serious imbalances may occur in the rations of livestock maintained on crops from relatively infertile soils. These imbalances may result from mismanagement contributed to by the inability of infertile soil to support legumes and large yields of nonleguminous crops. The use of legumes in the ration helps balance the rations of herbivorous animals. Large yields of grains and other nonleguminous crops provide adequate amounts and quality of ration for efficient livestock production.

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VARIATION IN SOME FACTORS AFFECTING THE LENGTH OF CALVING INTERVALS[1]

N. C. BUCH, W. J. TYLER, AND L. E. CASIDA
Departments of Dairy Husbandry and Genetics, University of Wisconsin, and Dairy Husbandry Research Branch, USDA, Madison, Wisconsin

SUMMARY

The causes of prolongation of the calving interval were studied for the intervals following 395 calvings of an outbred experimental Holstein-Friesian herd. Losses of time were attributed to one or more of the following conditions: (a) postpartum anestrus, (b) interruption of the estrual cycle, (c) out-of-breeding condition, (d) infertile service, and (e) loss of pregnancy. When averaged over the entire herd, these amounted to 0.3, 8.0, 5.2, 21.2, and 11.9 days, respectively. Cows for which it was necessary to delay breeding until the uterus attained breeding condition showed greater loss due to infertile service than did cows that did not require delay in breeding. More time also was lost due to out-of-breeding condition following abnormal, than following normal, calvings and by pluriparous, than by primiparous, cows.

Losses in breeding efficiency of dairy cattle usually can be attributed either to failure of the cows to be bred or to their return to heat after breeding. An unsatisfactory condition of the genital tract at the time of intended breeding may result in intentional delay in breeding. Also, cows may be late in returning to heat after calving or they may exhibit a break in estrual rhythm which causes delay. Some cows, though showing regular estrus, may not settle on first service when bred and repeat one or more times. Some cows that conceive lose the pregnancy, so that intervals between two calvings are prolonged.

The present paper deals with some of the factors that contribute to the total loss of breeding time and their relative importance. Their incidence and magnitude also have been studied in relation to parity and season.

MATERIALS AND METHODS

The data for this study were collected from the experimental herd of Holstein-Friesian cows at the Emmons Blaine, Jr. Experimental Farm, Lake Mills, Wisconsin. The cows in this herd are bred back at the first heat occurring on or after 75 days postpartum, provided they have been approved from rectal palpation of the genital organs as being in satisfactory breeding condition. The number of days between 75 days postpartum and the date of conception that was maintained 150 days or longer was considered as time lost.

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The standard procedure for culling cows from the herd because of loss of time is to eliminate them after 233 open days following the 75 days postpartum interval. In the event that a cow lost a diagnosed pregnancy before the 150th day of gestation, the number of days that it was pregnant was not counted toward the 233 open days, and it remained in the herd to complete 233 open days before being culled.

The procedures for collecting data on the various phases of reproduction were the same as described by Kidder *et al.* (3). The management factors and procedure for checking animals for heat were the same as described by Buch *et al.* (1).

The calving intervals following 311 normal calvings of outbred cows, as well as those following 84 abnormal calvings occurring between January 1, 1949, and December 31, 1954, were included for the study of total loss of time between calvings or from calving to the time the cow went out of the herd. The total loss of time was attributed to one or more of the following conditions: (*a*) postpartum anestrus; (*b*) estrual hiatus, or interruption of the estrual cycle; (*c*) out-of-breeding condition; (*d*) infertile service, and (*e*) loss of pregnancy.

As indicated above, the anestrual condition was classified into two types: (*a*) the prolonged postpartum anestrual condition, hereinafter referred to as postpartum anestrus, and (*b*) estrual hiatus, which is defined as a prolongation of the interval between two heat periods beyond a normal cycle length (21 days). If the animal showed an estrus before 75 days postpartum at such a time that the next heat would occur some time after the 75th day postpartum, the number of days lost (within the limits of the 21-day estrual cycle) was considered as loss due to chance and the remainder, if any, as loss due to estrual hiatus. In this study, the total loss of time does not include the number of days lost due to chance.

The time lost by a cow from 75 days postpartum until its approval for breeding was charged to the out-of-breeding condition. A prebreeding examination is made on all cows 68 to 74 days postpartum. If the animal is not found in a satisfactory breeding condition, the rectal palpations are continued regularly every week until it is in a satisfactory breeding condition. The cow is considered in a satisfactory breeding condition if the tone, consistency, size, and location of the uterus and cervix are found normal on palpation and no abnormal discharge is shown.

Loss of time ascribed to infertile services is defined as the time from first breeding to conception, provided the animal remained in a satisfactory breeding condition throughout the service period and was bred at all the heat periods following the first breeding. If the animal showed any other condition simultaneously with the out-of-breeding condition, the latter was given precedence in the classification of the data.

The pregnancy examination is made from 35 to 41 days after breeding on all cows not returning to estrus. If the animal lost a pregnancy after it was diagnosed pregnant, and before 150 days from the date of conception, the loss of time between the breeding that produced the initial diagnosed conception and

the breeding that produced the pregnancy carried at least 150 days was charged to loss of pregnancy. A further breakdown of these intervals was made into pregnancy duration and losses due to out-of-breeding condition and infertile service following the loss of pregnancy.

The years under study were divided into four calving seasons and the cows were divided into two parity groups: (*a*) primiparous, and (*b*) pluriparous. Cows that had calved only once were designated as primiparous and those that had calved twice or more were considered pluriparous [terms as used by Williams (7)].

All calvings were considered normal except those involving multiple births, retained placenta, dystocia, prolapse of the uterus, a dead calf, or an abortion (live or dead calf) occurring between 150 and 240 days from the date of conception. The conditions contributing to the total loss of time were studied following 84 abnormal calvings. However, the intervals following these calvings were not studied for the effect of parity or season.

The tests of significance for the effect of parity, season, and interaction between parity and season on the proportion of cows showing different conditions contributing to the loss of time were made by a method appropriate to the analysis of Chi-square as described by Rao (4). This method takes into account the unequal subclass numbers.

The lengths of various kinds of intervals contributing to the total loss of time were analyzed for the effect of season, parity, and interaction between parity and season for those cows calving normally, by the method described by Snedecor (5), and a subsequent correction of that method (6).

The importance of each of the conditions contributing to the total loss of time by the cows was determined both for those calving normally and for those calving abnormally, by calculating the standard partial regression coefficients as outlined by Wright (8).

RESULTS

The proportions of the intervals (in percentages) following 311 normal calvings in which the cows showed losses were: due to postpartum anestrus, 2; estrual hiatus, 59; infertile services, 38; out-of-breeding condition, 9, and loss of pregnancy, 10. The proportions of these conditions, and the average lengths of time wasted by those cows showing these conditions, are presented in Table 1. More than one of the conditions contributing to the total loss of time could be shown by the same cow at different times.

The average time lost by those cows showing various conditions was 15, 13, 55, 33, and 118 days, due to postpartum anestrus, estrual hiatus, infertile services, out-of-breeding condition, and loss of pregnancy, respectively (Table 1).

The lengths of time lost due to various conditions were not affected by season and parity, except that there was a significant difference in the mean lengths of time lost in the two parity groups (28 days for the primiparous and 36 days for the pluriparous) when the animals were in the out-of-breeding condition (Table 1).

TABLE 1
Conditions causing loss of breeding-time following normal and abnormal calvings

Season and normality of calving	Parity	No. of cows	Cows showing various conditions: P. P. anestrus % of all cows	P. P. anestrus Av. days lost	Estrual hiatus % of all cows	Estrual hiatus Av. days lost	Infertile services % of all cows	Infertile services Av. days lost	Out-of-breeding condition % of all cows	Out-of-breeding condition Av. days lost	Loss of pregnancy % of all cows	Loss of pregnancy Av. days lost
Normal:												
Winter	Primips[a]	39	3	7	51	7	36	40	13	31	8	96
	Plurips[b]	57	5	4	58	7	33	55	7	40	5	144
Spring	Primips	45	0	0	71	23	42	76	2	20	11	99
	Plurips	55	2	37	60	10	33	49	13	29	15	133
Summer	Primips	19	5	32	53	8	32	34	0	0	15	169
	Plurips	45	0	0	56	15	42	49	7	34	13	109
Autumn	Primips	19	0	0	63	25	37	60	16	25	5	72
	Plurips	32	0	0	63	7	50	63	19	42	9	93
Normal:												
Winter	Primips and Plurips	96	4	5	55	7	34	48	9	35	6	120
Spring	Primips and Plurips	100	1	37	65	16	37	63	8	28	13	119
Summer	Primips and Plurips	64	2	32	55	13	39	45	5	34	14	129
Autumn	Primips and Plurips	51	0	0	63	14	45	62	18[c]	36	8	88
Normal: All seasons	Primips	122	2	20	61	17	38	57	7	28	10	113
	Plurips	189	2	12	59	10	38	53	11	36[c]	11	121
Normal: All calvings		311	2	15	59	13	38	55	9	33	10	118
Abnormal: All calvings		84	4	15	65	14	44	51	18	74	11	117

[a] Primiparous.
[b] Pluriparous.
[c] .05>P>.01.

Since the incidence of loss due to postpartum anestrus was very small (2%), no statistical analysis was made of possible causes of its variation.

Examination of the data was made for the possible effects of the different factors on the proportion of cows showing various conditions contributing to the total loss of time. No statistically significant differences were observed between any of the sources of variation, except for the out-of-breeding condition, in which such a difference was observed between seasons. It can be seen (from Table 1) that the largest proportion of cows (18%) showed this condition during autumn. There was no interaction between parity and season.

The correlation coefficients between the various factors causing loss of time were practically zero, except that there was a highly significant ($P<0.01$) correlation of .24 between infertile services and out-of-breeding condition (Table 2).

TABLE 2

Correlation coefficients between the various conditions contributing to loss of time and total loss of time

	Estrual hiatus	Infertile services	Out-of-breeding condition	Loss of pregnancy	Total time
			Normals		
Postpartum anestrus	−.03	−.02	−.03	.04	.05
Estrual hiatus		−.03	−.06	−.04	.31
Infertile services			.24[b]	.02	.65
Out-of-breeding condition				.09	.35
Loss of pregnancy					.67
			Abnormals		
Postpartum anestrus	−.08	−.06	−.06	−.05	−.06
Estrus hiatus		.10	.12	−.05	.30
Infertile services			.25[a]	−.02	.65
Out-of-breeding condition				−.01	.64
Loss of pregnancy					.52

[a] $.05>P>.01$.
[b] $P<.01$.

However, the other nonsignificant correlation coefficients were used in calculating the standard partial regression coefficients of total time lost on each of the factors. These regression coefficients, and the variances for each of these factors following normal and abnormal calvings, are shown in Table 3.

A study of 84 abnormal calving intervals indicated that loss of time for the various conditions was 15, 14, 51, 74, and 117 days, respectively, for the cows showing postpartum anestrus, estrual hiatus, infertile services, out-of-breeding condition, and loss of pregnancy (Table 1). The correlation coefficients between the various factors were practically zero, except that there was a significant ($P<0.05$) correlation of .25 between the infertile services and out-of-breeding condition (Table 2).

TABLE 3

Relative importance of factors contributing to loss of time after normal and abnormal calvings and variances of the losses of time due to the various factors

	Normal calvings		Abnormal calvings	
Condition	Standard partial regression	Variance	Standard partial regression	Variance
Postpartum anestrus	0.04	8	0.04	12
Estrual hiatus	0.37	513	0.22	276
Infertile services	0.60	1,366	0.52	1,538
Out-of-breeding condition	0.17	113	0.49	1,366
Loss of pregnancy	0.65	1,593	0.55	1,720
Total (all conditions)		3,778		5,718

DISCUSSION

The present study emphasizes the relative importance of different sources of variation in loss of time, from calving to conception which holds at least 150 days. From the standpoint of the proportion of normally calving animals that lost time for the different causes (Table 1), the incidence of estrual hiatus was the highest (59%) and infertile services next (38%), whereas that of the postpartum anestrus was the lowest (2%). The estimate of the incidence of estrual

hiatus may be considered as excessive, inasmuch as it included all intervals over 21 days. Any limit used had to be more or less arbitrary and the modal length appeared as good as any. The incidence of out-of-breeding condition and of pregnancies lost between diagnosis and 150 days from the date of conception was relatively low, 9 and 10%, respectively. Although the incidence of estrual hiatus was highest, from the standpoint of the individual affected animal, the most serious causes in terms of the number of days lost were infertile services (55 days) and loss of pregnancy (118 days). Following a loss of pregnancy, it was found that the major contribution to the total loss of time was the animal remaining in an out-of-breeding condition. This subsidiary analysis is not shown in a table, but the incidence of out-of-breeding condition was 73% and that of infertile services was 34% following loss of pregnancy.

Following abnormal calvings, the incidence of conditions other than out-of-breeding condition was very close to that following normal calvings (Table 1). The incidence of out-of-breeding condition (18%) was higher than that found in cows calving normally (9%). There were 41 more days lost by the animals showing out-of-breeding condition following abnormal calvings than by similar cows following normal calvings. Thus, out-of-breeding condition, when both incidence and duration are considered, appears to be approximately as important as infertile services and loss of pregnancy in determining loss of time following abnormal calvings. Johansson (2) reported that the calving intervals following twinning were increased in length, probably due to retention of afterbirth and endometritis.

It might be assumed that this herd has a proportion of normal and abnormal calvings similar to that of many herds. Calculations were made of the losses of time due to the different causes when averaged over all the cows in the herd (Table 4). These values indicate the actual prolongation of the average calving interval due to the different causes. Infertile services and loss of pregnancy together were responsible for more than 1 mo. in average time lost.

Finally, from the standpoint of variability, the cows calving normally had variances in loss of time due to infertile services and loss of pregnancy of 1,366 and 1,593, respectively, as compared to 513 and 113 due to estrual hiatus and out-of-breeding condition (Table 3). For abnormal calvings, the variance for loss of time due to out-of-breeding condition, 1,366, was more nearly comparable to infertile services and loss of pregnancy,1,538 and 1,366. The standard partial

TABLE 4

Prolongation of average calving interval in the herd, due to different losses of time

Source of loss	No. days lost, averaged over whole herd
Postpartum anestrus	0.3
Estrual hiatus	8.0
Out-of-breeding condition	5.2
Infertile services	21.2
Loss of pregnancy	11.9
Total	46.6

regression coefficients for the losses due to these factors are indicative of their relative importance.

The correlations of .24 and .25 (Table 2) between losses of time due to out-of-breeding condition and to infertile services, for the normal and abnormal intervals, respectively, may have a cause-and-effect implication. More detailed examination brought out one pertinent relationship. Both normal and abnormal cows that were still in out-of-breeding condition at 75 days postpartum subsequently showed a higher incidence of loss due to infertile services than did cows that were in breeding condition at 75 days.

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EFFECT OF SOME GENETIC AND MATERNAL ENVIRONMENTAL VARIATIONS ON BIRTH WEIGHT AND GESTATION LENGTH IN HOLSTEIN CATTLE [1]

W. D. FOOTE, W. J. TYLER, AND L. E. CASIDA
Departments of Genetics and Dairy Husbandry, University of Wisconsin, and Dairy Cattle Research Branch, USDA, Madison, Wisconsin

SUMMARY

Records on 536 gestations from 258 Holstein cows representing six different sire lines were studied by standard partial regression and analysis of variance. Birth weight and gestation length were analyzed for possible effects of sire line, inbreeding and sex of calf, and weight, inbreeding, parity, and uterine horn pregnant in the dam.

The average inbreeding of inbred dams was 24.7% and of inbred calves, 24.9%. Inbreeding of the dam had insignificant effects both on birth weight and on gestation length. The standard partial regression of birth weight on inbreeding of calf was negative and statistically significant. Inbreeding of the calf, however, had no significant effect on gestation length. Both birth weight and gestation length increased with the parity order of the dam, but the effect was significant only in the case of birth weight. Weight of dam had a positive effect ($P < 0.05$) on both dependent variables. There were significant differences between sire lines both in birth weight and in gestation length. Male calves averaged 5.92 lb. heavier at birth and were gestated 1.44 days longer than females. The right uterine horn produced heavier calves (av. 1.78 lb.; $P < 0.05$) than the left horn, but the side pregnant had no significant effect on gestation length. Interactions between the effects of sire line, sex of calf, and uterine horn pregnant were also tested and none was significant.

Breed, sire of fetus, and weight and age of dam were shown by Eckles (5) (1919) and Fitch *et al.* (7) (1924) to affect birth weight and gestation length in dairy cattle. Jafar *et al.* (10) (1950) estimated that the genotype of the calf within sexes, and the permanent maternal characteristics, were responsible for 48.0 and 21.0%, respectively, of the variation in gestation length of Holstein-Friesian cattle. Work by Knoop and Hayden (12) (1934), Brakel *et al.* (1) (1952), Herman *et al.* (8) (1953), and Rollins *et al.* (15) (1956) indicates that gestation length increases until the dam reaches maturity. Fitch *et al.* (7) (1924) and Davis *et al.* (3) (1954) have reported similar trends for birth weight. Eckles (5) (1919) found a consistent positive relationship between the mature weight of the dam and of the calf within the Holstein breed. Dickerson (4) (1940) found calves averaging 16% inbred to average nearly 10% lighter at birth than outbred calves by the same sire. Knott (13) (1932) and Jafar *et al.* (10) (1950) have

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shown male calves to be carried longer in utero than female calves. Eckles (5) (1919) and Tyler *et al.* (17) (1947) have shown that males are generally heavier than females of the same breed.

The possibility of local factors within the maternal environment affecting fetal development has been examined in guinea pigs and in swine [Ibsen (9), 1928; Eckstein and McKeown (6), 1955; Waldorf *et al.* (18), 1957]. However, in their studies, competition between fetuses was considered to be a factor in maternal environment, which usually would not be true for cattle. The observation of greater activity of the right than of the left ovary in the cow [Clark, (2) 1936; Reece and Turner, (14) 1938, and Kidder *et al.*, (11) 1952], and the consequent majority of right-sided pregnancies, have suggested a need to test for possible asymmetrical activity of reproductive organs other than the ovaries, such as the uterine horns.

The birth weights and lengths of gestation periods from a controlled breeding experiment have been studied to determine the effects of some genetic and maternal environmental variables; namely, sire line of the dam, inbreeding and sex of the calf, inbreeding of the dam, parity and weight of the dam, and uterine horn pregnant in the dam (i.e., whether the fetus was carried in the right or left uterine horn).

MATERIALS AND METHODS

The data studied were furnished by the Emmons Blaine, Jr., Holstein-Friesian herd at Lake Mills, Wisconsin. This experimental breeding herd was established in 1948, with six proven bulls and one or two outbred sons and 20 open outbred daughters from each of these bulls. By systematic mating of sire to daughter or brother to sister and of sire to unrelated female, six distinct sire lines representing these six original bulls have been developed, which include both inbred and outbred animals. Sire line as used in this report refers to the sire line of the dam of the calf. Since all of the animals are under the same management program, all lines are subjected equally to most environmental factors which may affect reproductive performance. Therefore, this herd can be expected to provide valid information regarding the effect of line as a genetic influence on the various aspects of reproduction.

Possible effects of uterine horn pregnant on fetal development as measured by calf birth weight and gestation length were tested. The uterine horn in which each calf was gestated was determined by rectal palpatation of each dam when it was from 35 to 41 days pregnant.

The association of inbreeding of dam, inbreeding of calf, parity of dam, and weight of dam with both gestation length and birth weight was estimated by within-line correlation, partial regression, and standard partial regression. Each gestation period and birth weight was adjusted for the linear influence of these variables by using the calculated partial regressions as adjustment factors, before the data were analyzed for effects of line, sex of calf, and uterine horn pregnant.

In classifying the data for calculation of the above-mentioned associations, the dams and calves were considered either outbred ($F_x = 0$) or inbred ($F_x > 0$).

The per cent inbreeding of inbred dams ranged from 13 to 39 and averaged 24.7, whereas these values for inbred calves were 13, 46, and 24.9, respectively.

In determining the parity order number of normal calvings, any pregnancy carried longer than 150 days was considered a calving. Parity was classified as first, second, and later-than-second calvings.

Weights of dams 3 mo. after calving were placed into three groups representing roughly the lower, middle, and upper thirds of the weight range. One hundred and one dams' weights were less than 1,050 lb., 349 were 1,050 to 1,350, and 86 were over 1,350 lb.

Records from 258 cows with 536 gestations terminating in live births of single calves from January, 1948, through December, 1955, were studied.

RESULTS AND DISCUSSION

The unadjusted gestation periods ranged from 258 to 293 days and averaged 278.2 days. The range and average for unadjusted birth weights were from 49 to 129 lb. and 91.8 lb., respectively.

Correlations between all combinations of the variables for which adjustments were made are given (Table 1). These correlations are peculiar to this herd because of the management plan in carrying out the breeding program. An association between inbreeding of dam and inbreeding of calf ordinarily might be expected. The insignificant association between inbreeding of dam and inbreeding of calf noted here ($r = 0.039$) could be due to the system of mating being designed to produce a desired number of both inbred and outbred calves from inbred and outbred dams. The significant negative correlations between inbreeding of dam and both parity and weight of dam ($r = -0.138$ and -0.224, respectively) are to be expected, since the herd began with outbred animals only. Thus, the average outbred dam was older and, consequently, had more calvings and heavier body weight. The correlations between inbreeding of calf and parity ($r = 0.185$), and between inbreeding of calf and weight of dam ($r = 0.089$), indicate that the older and, consequently, heavier cows having more calving sequences were bred to produce inbred calves. The highly significant correlation between parity and weight of dam ($r = 0.608$) is to be expected, since both tend to increase with age.

The correlation between the variables just discussed and the dependent variables, birth weight and gestation length, are given in Table 2, with the corre-

TABLE 1

Within-family line correlations of independent variables

Variables	r
Inbreeding of dam — Inbreeding of calf	0.039
Inbreeding of dam — Parity	−0.138[a]
Inbreeding of dam — Weight of dam	−0.224[a]
Inbreeding of calf — Parity	0.185[a]
Inbreeding of calf — Weight of dam	0.089[b]
Parity — Weight of dam	0.608[b]

[a] $P < .01$.
[b] $P < .05 > .01$.

TABLE 2
Associations between independent variables and birth weight and gestation length

Independent variables	Birth weight			Gestation length		
	r	b′[c]	b[d] *(lb.)*	r	b′[c]	b[d] *(days)*
Inbreeding of dam	−.092	.005	0.140	.037	.077	0.922
Inbreeding of calf	−.192	−.269[a]	−6.534	.077	.047	0.496
Parity	.389[a]	.326[a]	4.734	.160[a]	.078	0.485
Weight of dam	.559[a]	.186[b]	3.843	.172[a]	.138[a]	1.214

[a] $P < .01$.
[b] $P < .05 > .01$.
[c] Standard partial regression.
[d] Partial regression.

sponding standard partial regressions and partial regressions. The correlations themselves are influenced by the associations among the independent variables discussed above. Major attention, therefore, will be given to the standard partial regressions and partial regressions as the best indication of the direct influence of these variables on birth weight and gestation length. Inbreeding of the dam had no real effect either on birth weight or on gestation length ($b' = 0.005$ and 0.077, respectively). The negative regression of birth weight on inbreeding of calf was significant ($b' = -0.279$ and $b = -6.53$ lb.). Insignificant associations were found between gestation length and inbreeding of calf ($b' = 0.047$) and between parity and gestation length ($b' = 0.078$). However, birth weight increased as the calving sequences advanced ($b' = 0.326$). The partial regression showed birth weight to increase 4.73 lb. for each succeeding calving sequence studied. Weight of the dam had a significant effect on both birth weight and gestation length ($b' = 0.186$ and 0.138, respectively). The partial regressions for birth weight and gestation length on weight of dam were 3.84 lb. and 1.21 days per dam weight group.

All birth weights and gestation lengths were adjusted for their partial regressions on inbreeding of both fetus and dam, parity, and weight of dam, even though their effects were found not to be statistically significant in many of the cases.

The means of the adjusted birth weights and gestation lengths are shown (in Tables 3 and 4, respectively). Analysis of these data, using the method of unweighted means [Snedecor, (16) 1956] to test for main effects and interactions of sire line, sex of calf, and uterine horn pregnant on two dependent variables, is shown in Table 5. Genetic influences both on birth weight and on gestation length are evidenced by the highly significant differences between sire lines for both variables. Also, male calves were significantly heavier at birth than females (5.92 lb.; $P < 0.01$) and were carried significantly longer (1.44 days; $P < 0.05$). Calves carried in the right uterine horn were significantly heavier than those in the left (1.78 lb.; $P < 0.05$). There was no significant difference in gestation lengths between uterine horns. It is suspected that the difference in birth weights due to uterine horn pregnant more definitely is due to differences in prenatal growth rate than to variations in length of prenatal period. However, a direct test was not made of this point. No significant interactions were

TABLE 3

Mean adjusted birth weights by sire line, sex of calf, and uterine horn

Horn	Sex	Line					
		Hartog	Netherland	Mooie	Lalaur	Ollie	Belmont
		(lb.)					
Right							
	Male	88.9 (18)[a]	79.7 (26)	89.3 (19)	84.4 (22)	81.6 (31)	83.4 (33)
	Female	79.8 (25)	77.3 (17)	84.1 (18)	76.9 (31)	72.5 (36)	78.6 (27)
Left							
	Male	82.8 (23)	75.9 (16)	90.0 (17)	83.8 (15)	77.5 (15)	84.1 (17)
	Female	81.7 (19)	77.3 (23)	79.5 (21)	75.5 (28)	68.9 (18)	78.3 (21)
Line means:		83.3 (85)	77.6 (82)	85.7 (75)	80.2 (96)	75.1 (100)	81.1 (98)

Horn means: Right—81.4 (303); left—79.6(233)
Sex means: Male—83.4 (252); female—77.5 (284)

[a] Numbers of calves are in parentheses.

found between any combinations of line, sex of calf, and horn pregnant for either birth weight or gestation length.

DISCUSSION

Any influence of sex and inbreeding of calf would be expressed as an influence of the genotype of the fetus on fetal development, either on rate of growth or length of growth period. The latter effect seems unlikely, at least for inbreeding of fetus, since this inbreeding had no significant effect on gestation length. Differences in the genotype of the dam as expressed by the maternal environment afforded her fetus, and in her genetic contribution to the fetus, could contribute to the effect of sire line. This influence can not be considered to have its effect through some other factor for which adjustment was made, such as weight of dam. Weight and inbreeding of dam may be considered both as genetic and as maternal environmental variables acting on fetal development. Heavier dams may produce heavier calves by virtue of having greater facilities for fetal growth or because of a genetic association between adult weight and birth weight. The influence of inbreeding of dam on both dependent variables was negligible. This might suggest that neither the natural selection exerted on the dam's genotype consequent to inbreeding, nor the environment it provides due to being inbred, affects fetal

TABLE 4

Mean adjusted gestation length by sire line, sex of calf, and uterine horn

Horn	Sex	Line					
		Hartog	Netherland	Mooie	Lalaur	Ollie	Belmont
Right							
	Male	274.7[a]	274.4	275.4	276.6	277.0	276.3
	Female	270.7	274.3	275.5	275.6	275.5	276.6
Left							
	Male	275.8	274.2	275.7	278.0	277.9	279.3
	Female	274.6	274.0	275.1	275.3	274.2	276.4
Line means:		274.0	274.2	275.4	276.4	276.2	277.2

Horn means: Right—275.2; left—275.9
Sex means: Male—276.3; female—274.8

[a] Days. Numbers of calves in the subgroups are the same as in Table 3.

TABLE 5

Analysis of variance of unweighted means of birth weights and gestation lengths

Source	DF	Error term	Birth weight M.S.	Gestation length M.S.
Sire line (L)	5	W	1,236[a]	133[a]
Sex of calf (S)	1	L × S	4,398[a]	259[b]
Uterine horn (H)	1	L × H	396[b]	48
L × S	5	W	196	26
S × H	1	L × S × H	24	25
L × H	5	W	37	26
L × S × H	5	W	108	23
Within subclass (W)	512		115	24

[a] $P < .01$.
[b] $P < .05 > .01$.

development appreciably. An exception to this would be if both actions were real but opposite in direction, cancelling each other.

Parity, found to affect only birth weight, would appear to have its effect solely through maternal environment. The action of each gestation and increasing age may be to prepare the reproductive system to provide a better environment for subsequent gestations. The basis of the effect of uterine horn, another influence of maternal environment, is not known. A local influence of differential ovarian activity seems unlikely, since ovarian secretions apparently are released systemically. However, ovarian and uterine asymmetry of function could be due to a common cause. Explanations such as crowding of the pregnant left horn by rumen, or of differential blood supply to the uterine horns, are without evidence.

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A STUDY OF SOME POSSIBLE GENETIC CAUSES OF MUMMIFIED FETUSES IN DAIRY CATTLE

O. W. DEATON, DURWARD OLDS, AND D. M. SEATH
Kentucky Agricultural Experiment Station, Lexington

SUMMARY

A total of 23 mummified fetuses was recorded among 1,509 pregnancies of 504 cows. Thus, mummified fetuses were produced by 3.7% of the cows and in 1.5% of the observed pregnancies. It was found that 29.9% of the pregnancies resulted in mummified fetuses in those cows which produced mummies. On the other hand, of the 20 bulls which were known to have sired mummies, only 4.2% of their offspring were mummies. Chi-square values, used to test the closeness of fit of various observed ratios to expected Mendelian ratios regarding the occurrence of normal males and females and mummified fetuses, indicated that the mummification process is not due to a homozygous autosomal recessive gene but may be due to a sex-linked recessive lethal gene.

Mummification of the bovine fetus commonly has been attributed to an autosomal recessive lethal (3, 5, 6, 7). However, Boyd (1) has described one case in which torsion of the uterus appeared to be the cause of mummification. Erb and Morrison (2) recently have reported a study of 32 mummified fetuses, in which they concluded that the condition is not due to a single recessive lethal and that environmental influences very likely are involved. Hornaday (4) found that in some herds as much as 5% of the breeding females produced mummified fetuses. He also postulated that the lethal might be sex-linked, since the abnormality was observed in some cow families for several generations. Moutiejunas, as cited by Williams (8), observed five mummified fetuses and found them all to be males.

According to Roberts (6), bovine fetal mummification may occur from the 3rd to the 8th mo. of gestation, and usually is accompanied by slight to severe interplacental hemorrhage, causing a separation of the maternal and fetal placentas. The fetal membranes wrap around the fetus, and the wall of the uterus firmly encloses the fetus, the fetal membranes, and a variable amount of gummy, reddish-brown material which stains both the membranes and the fetus. The longer the condition exists, the drier, firmer, and more leather-like the tissues of the fetus become. Although abortions may take place, the condition usually is not suspected until parturition fails to occur.

The present study was made for the purpose of evaluating further the likelihood that mummification of the fetus is a genetic lethal, and to determine, if possible, the mode of inheritance.

METHODS

Breeding records for the Kentucky Agricultural Experiment Station dairy herd were studied covering a period of 39 yr. (1919 to 1958). All calvings were tabulated according to sires and dams. Unadjusted Chi-square values were used

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

to test the closeness of fit of various observed ratios to expected Mendelian ratios, regarding the occurrence of normal calves and mummified fetuses.

RESULTS

A total of 23 mummified fetuses was recorded among 1,509 pregnancies of 504 cows. One cow produced three mummies, and two cows each produced two mummies. Thus, it may be calculated that 1.5% of the observed pregnancies resulted in mummified fetuses. Likewise, 3.7% of the cows produced one or more mummies during their lifetime. The frequency of mummification was the same in both the Holstein and Jersey breeds.

The 19 cows which produced mummies had a total of 77 parturitions. Thus, 29.9% of the observed pregnancies in these cows resulted in mummified fetuses. On the other hand, there were 20 bulls known to have sired mummies and only 4.2% of their offspring were mummies.

The cows producing mummified fetuses required an average of 1.84 services per conception for all of their pregnancies. Those pregnancies resulting in mummified fetuses required only 1.57 services per conception.

Mating of bulls that have sired mummies, to cows that have borne mummies, would be expected to produce 25% mummies, if the condition is caused by an autosomal recessive gene. Of 43 such carrier × carrier matings in this herd, there were actually 20 normal calves and 23 mummified fetuses. The Chi-square value of 17.6 indicated that there is very little likelihood that the actual 20:23 ratio would be observed if the true ratio should be 3:1. Therefore, it is unlikely that mummification of the fetus in this herd was due to an autosomal recessive lethal.

Theoretically, half of the cows with one carrier parent would themselves be carriers. If such cows (carriers and noncarriers) were mated to carrier bulls, 12.5% of the offspring would be expected to be mummies (7:1 ratio). Actually, the observed ratio for these matings in this herd was 114:3. Again, Chi-square indicated that the lethal was not caused by a single autosomal recessive gene.

Considering the possibility that mummification could be due to a sex-linked lethal gene, it is postulated that all mummies would be males, all living males would be normal, and only cows could be carriers. In this case, the normally expected ratio for the offspring of known carrier cows would be 1 male: 2 females: 1 mummy. Of 74 such pregnancies, there were 21 males, 30 females, and

TABLE 1

Summary of data regarding observed and expected ratios when mummification is assumed to be caused by a homozygous autosomal recessive gene in the fetus

Kind of mating		Actual and expected ratios	
		Normal	Mummified
Carrier x carrier	Actual	20	23
	Expected	32	11
	Chi-square=	17.6[a]	
Carrier x daughters of carriers	Actual	114	3
	Expected	102	15
	Chi-square=	10.5[a]	

[a] $P<0.01$, indicating that mummified fetuses are probably not caused by autosomal recessive genes.

TABLE 2

Summary of data regarding observed and expected ratios when mummification is assumed to be caused by a sex-linked lethal gene

Kind of mating		Actual and expected ratios		
		Males	Females	Mummies
All bulls x carriers	Actual	21	30	23
	Expected	18.5	37	18.5
	Chi-square=	2.75 N.S.[a]		
All bulls x daughters of carrier cows	Actual	25	28	3
	Expected	21	28	7
	Chi-square=	3.1 N.S.[a]		

[a] Since the Chi-square values were not significant, it appears that mummification could reasonably be due to a sex-linked lethal recessive gene.

23 mummified fetuses. The Chi-square value of only 2.75 indicated the probability that mummification could well be caused by a sex-linked lethal gene.

If mummification is due to a sex-linked gene, then one-half of the daughters of carrier cows would also be carriers. Therefore, the daughters of carrier cows would be expected to produce offspring in the ratio of 3 males: 4 females: 1 mummy. Among 56 such pregnancies, there were actually 25 males, 28 females, and 3 mummified fetuses. Again, the Chi-square value of 3.0 indicated that sex-linked inheritance is a possibility.

Summaries of these tests for suitability of fit are given (Tables 1 and 2).

DISCUSSION

The data presented in this study seem to indicate rather conclusively that mummification of the fetus is not caused by a homozygous recessive autosomal gene. On the other hand, it is entirely possible, though not conclusively established, that mummification is due to a sex-linked lethal gene. There should be great interest in observing the sex of any mummified fetuses which occur in the future. If any of them are females, that would seem to rule out the present theory of sex-linkage. Also, it is possible that planned matings could be arranged and results noted through the cooperation of several herds. It would be of both practical and academic interest to determine more about the physiological mechanism of mummification. For example, does the interplacental blood originate from the fetus, the dam, or both? What initiates this flow of blood?

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COMPARISON OF MATES OF SIRES USED ARTIFICIALLY AND NATURALLY IN MICHIGAN DHIA HOLSTEIN HERDS [1, 2]

V. C. BEAL, JR.,[3] AND D. E. MADDEN
Department of Dairy, Michigan State University, East Lansing

SUMMARY

Michigan DHIA Holstein records from 1944 to 1956 were analyzed to compare production levels of mates of sires used artificially (AB) and naturally (non-AB).

Purebred and grade mates were analyzed separately, since the former had higher levels of production and a higher average number of records than the latter. Purebred mates of AB sires did not produce significantly more milk or fat but did have significantly more records than non-AB purebred mates (12,794 lb. milk, 452 lb. fat, and 2.79 records for AB mates, and 12,711 lb. milk, 449 lb. fat, and 2.60 records for non-AB mates). Grade mates of AB sires did not produce significantly less milk or fat or have significantly more records than non-AB grade mates (11,537 lb. milk, 421 lb. fat, and 2.43 records for AB mates, and 11,659 lb. milk, 422 lb. fat, and 2.34 records for non-AB mates). Since AB and non-AB sires were mated to equally productive mates, and other workers have found no difference between AB and non-AB daughters, this study supports the conclusion that significant differences in transmitting ability have not been present between groups of AB and non-AB sires studied.

Several workers (1, 3) have reported no difference between contemporary AB and non-AB daughters. These workers did not report on differences between the dams of the daughters used in their studies. Since differences in levels of mates of AB and non-AB sires might alter the picture, the present study compared production levels of mates to ascertain if AB and non-AB mates differed and if dairymen mated cows of different production levels in specific methods, either artificially or naturally.

METHODS

Michigan DHIA Holstein milk and fat production records used in this study were obtained from approximately 65,000 records reported from 1944 to 1956. Mates of registered sires were classified as grade or purebred, the latter only when both the mate and its daughter were registered. Similarly, mates were classified as AB or non-AB mates when their daughters were sired by AB or by non-AB sires. About one-fourth of the mates appeared both as AB and non-AB mates. The average of all adjusted (2×−305 day–M.E.) records by a cow was used as the measure of its producing ability. Data were included for herds which had both AB and non-AB mates.

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[2] These data are part of a thesis presented by the senior author to the College of Agriculture, Michigan State University, in partial fulfillment of the requirements of the degree of Master of Science.

[3] Present address is Department of Dairy, Purdue University, Lafayette, Indiana.

RESULTS AND DISCUSSION

Average production levels for purebred and grade mates of AB and non-AB sires are presented (Table 1). Purebred mates of AB and non-AB sires differ little in average number of records and production levels. Grade mates of AB and non-AB sires differ little in average number of records and production levels, but are lower than the purebred mates.

TABLE 1

Average milk and fat production for purebred and grade mates of AB and non-AB sires

Source of data			Records		Milk (lb.)		Fat (lb.)	
Sires	Mates	No. of mates	Average No.	Standard deviation	Average	Standard deviation	Average	Standard deviation
AB	Purebred	1,055	2.89	1.70	12,623	2,322	448	84
Non-AB	Purebred	2,286	2.77	1.53	12,723	2,415	451	90
AB	Grade	126	2.52	1.59	11,736	2,143	422	71
Non-AB	Grade	187	2.39	1.55	11,966	2,096	432	76
Total		3,654	2.79	1.67	12,621	2,375	448	88

Because of the wide variation in numbers of comparisons within herds, an analysis of variance for two classifications and disproportionate subclass numbers using unweighted means (2) was chosen to test for differences between AB and non-AB mates. Herds with less than two mates in a Herd by Method of Breeding subclass were excluded when comparing mates, since these provided no information for estimating the error or within-subclass variance. Average number of records and production levels were obtained for AB and for non-AB mates in the same herd. These intra-herd AB and non-AB averages were then averaged over all herds and are presented in Table 2 as averages of intra-herd means. The mates do not differ and AB and non-AB grade mates do not differ, but purebred mates had more records and higher production than grade mates.

TABLE 2

Averages of intra-herd mean milk and fat production for purebred and grade mates of AB and non-AB sires

Source of data			Records		Milk (lb.)		Fat (lb.)	
Sires	Mates	No. of herds	Average No.	Standard deviation	Average	Standard deviation	Average	Standard deviation
AB	Purebred	183	2.79	1.02	12,794	1,876	452	69
Non-AB	Purebred	183	2.60	.82	12,711	1,713	449	63
AB	Grade	31	2.43	.95	11,537	1,678	421	59
Non-AB	Grade	31	2.34	1.08	11,659	1,628	422	60
Total		214	2.65	.94	12,585	1,819	446	66

Analyses of differences in number of records per cow between herds and between methods of breeding (AB vs. non-AB) are presented in Table 3. Highly significant differences between herds were obtained for both purebred and grade mates. Purebred AB mates had significantly more records per mate than did

TABLE 3

Analysis of differences in number of records and production for mates of AB and non-AB sires

Source of variation	Purebred mates				Grade mates			
	d.f.	Mean squares			d.f.	Mean squares		
		No. of records	Milk[a]	Fat		No. of records	Milk[a]	Fat
Herds	182	1.38[b]	5,631[b]	7,612[b]	30	1.59[b]	4,855[b]	6,579[b]
Methods of breeding	1	3.34[b]	628	959	1	0.10	232	7
Herds × methods	182	0.34	840	1,109	30	0.48	611	596
Error	2,975	0.43	779	1,026	251	0.48	765	950

[a] Milk multiplied by 10^{-3}.
[b] Significant at the 1% level.

their non-AB counterparts. The small difference (2.79 for AB and 2.60 for non-AB mates), although statistically significant, has little practical significance and indicates that purebred AB mates remain in the herd only slightly longer than purebred non-AB mates. Grade AB and non-AB mates did not differ significantly.

Differences between herds and between methods of breeding in levels of milk and fat production for AB and non-AB mates are presented in Table 3. Significant differences between herds were obtained for both purebred and grade mates. Mates did not differ in production level between the methods of breeding for either purebred or grade mates, nor were significant herd-by-methods interactions present. The absence of significant differences in productive levels between AB and non-AB mates indicates that AB and non-AB sires were mated to equally productive mates. Since the herd-by-method-of-breeding interactions were not significant, one must conclude that dairymen are not breeding their best cows in a given manner. Michigan dairymen, in response to a questionnaire (3), indicated that the grade herds are breeding their better cows artificially more frequently than are the registered herds, that some dairymen with both registered and grade cows may breed the former artificially and the latter naturally, and that the grade herds are breeding their heifers naturally more frequently than are the registered herds.

The present study provides only general information on these subjects. If Michigan dairymen are attempting to mate certain levels of their cows in a given manner, they have not been successful. Although their intentions may be to mate their best cows artificially, or vice versa, insofar as the average record is a good indication of the cow's ability, the dairymen have not actually recognized their better cows. The grade breeder is not breeding his better cows artificially any more often than the purebred breeder.

As mentioned previously, a dam may appear both as an AB and non-AB mate. The occurrence of such dams would reduce differences between AB and non-AB levels and supports the conclusion that dairymen actually are not selecting their better cows to mate in a given manner, even if they desire to do so.

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RELATIONS BETWEEN TEST-DAY MILK PRODUCTION OF HOLSTEIN COWS[1]

D. E. MADDEN, L. D. McGILLIARD, AND N. P. RALSTON
Department of Dairy, Michigan State University, East Lansing

SUMMARY

Milk production on test day from 6,715 Holstein-Friesian HIR 2 × and 3 × records during 1952–54 was studied, to determine the relations between production for single months and cumulative production, and to develop factors for extending part production to a 305-day basis.

Correlations between milk produced on test days were largest for adjacent months and ranged from .38 to .89 for 2 × — cows under 3 yr.; from .23 to .88 for 2 × — 3 yr. and over; from .39 to .89 for 3 × — under 3 yr., and from .21 to .88 for 3 × — 3yr. and over.

Correlations between milk production on test day, and the sum of milk produced on ten test days, ranged from .71 to .93, were higher for the young cows, were similar for different milking frequencies in the same age group, and were highest during the 4th to 7th mo. The regressions of the sum of production for ten test days on single test-day production had relationships similar to the corresponding correlations.

Factors developed from the ratio of total to cumulative part production, and from the regression of total on part production, are presented. The ratio method may underestimate total production of low-producing cows and overestimate total production of high-producing cows, since the ratio method corrects only for the incompleteness of the lactation and does not take into account the incomplete repeatability of the parts of the lactation, which is a part of the regression method. This difference is largest during the early months.

Biased evaluation of a sire's transmitting ability may result from the selection of daughters and/or their records. Programs for proving sires allow dairymen to determine at an early stage in lactation those cows which are unsatisfactory producers, and to remove these from their herds, in order not to lower the average production of a sire's daughters. If this procedure is common, and since injury and disease cause cows to leave herds prior to completion of lactations, perhaps some use should be made of information available in part lactations, which is not being utilized.

Associations between milk production at various stages in lactation have led workers (1, 4) to suggest that certain periods of the lactation may be more indicative of production for the entire lactation than are other periods. Several workers have developed factors for predicting total (305- or 365-day) production from single tests or cumulative parts of lactations (1, 5, 6, 9, 10). Few workers (6, 9, 10) have included age as an influence requiring joint consideration, although there has been general agreement for many years that younger cows are more persistent, but with lower maximum production, than older cows.

Relations between single test-day milk production and between cumulative

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part and total milk production were measured in this study. Factors were developed for extending incomplete production records to a 305-day basis. Age, milking frequency, and level of production were considered as influences which might affect the prediction factors obtained.

DATA AND METHODS USED

Monthly milk production recorded during 1952-54 for Holstein-Friesian Herd Improvement Registry (HIR) cows[2] throughout the United States was utilized. The first ten tests were used from lactations which had production recorded for at least ten different test days. Lactations with the same milking frequency (2× or 3×) throughout were utilized. Previous work (9) indicated that two age groups should be considered; namely, lactations which were initiated prior to 3 yr. of age and lactations initiated at 3 yr. and older. These same age groups have been used in the present study for several reasons: *(1)* When average monthly test-day production for year of age was plotted, the lactation curves for one- and two-year-olds were parallel, the curves for three-, four-, five-, and six-year-olds were parallel, but differed from the younger ages (one and two) by achieving a larger maximum but declining more rapidly, the curves crossing at about 9 mo. in production. *(2)* Average production on first test day for monthly age groups through 6 yr. of age differed significantly, and tests by Duncan's Multiple Range test (3) indicated means for ages under 3 yr.; and means for ages 3 yr. and older did not differ within groupings, i.e., overlapping within groups occurred, but overlapped between groupings only at 35 and 37 mo. of age. *(3)* When 3,930 Michigan DHIA–IBM Holstein records (8) were plotted by lactation number and age at freshening through 50 mo. of age (with age in months as the abscissa and per cent freshening at a given age for each lactation number as the ordinate), curves of the first and second lactation crossed between 35 and 36 mo. of age, and curves of the second and third lactation crossed between 47 and 48 mo. of age. That is, between 35 and 36 mo. of age the per cent of first lactations initiated was approximately equal to the per cent of lactations initiated. Twelve per cent of the second lactations were initiated prior to 36 mo. and 10% of the first lactations were initiated after 35 mo. Similar reasoning applies to the second and third lactations. Since HIR lactations would not be expected to start at an earlier age than DHIA lactations, and since the most important information lost pertains to incomplete first lactations, it seemed sufficient to proceed with the two age groups (under 3 yr., and 3 yr. and over).

RESULTS AND DISCUSSION

The number of lactations satisfying the above conditions are presented (in Table 1), with the corresponding average production on various test days. Approximately equal average production for the young and older groups is noted at the 9th mo., with the young cows exceeding the older cows at the 10th mo. Somewhat more variability is in the "under 3" group than in the "3 and over" group and in the 3× than in the 2×. Curves of average monthly production

[2] These data were provided by the Holstein-Friesian Association of America, Brattleboro, Vermont.

TABLE 1
Average test-day milk production[a]

Milking frequency	2 ×		3 ×	
Age	Under 3 yr.	3 yr. and over	Under 3 yr.	3 yr. and over
No. records	1,662	4,037	254	762
Test day	(lb.)			
1	41.3	56.7	46.3	59.1
2	42.0	57.0	47.0	61.5
3	39.3	52.4	45.0	57.5
4	37.1	47.6	42.9	52.5
5	35.3	43.6	40.9	48.5
6	33.7	40.0	39.6	45.2
7	32.3	36.9	38.5	42.1
8	30.6	33.3	36.4	38.6
9	28.4	29.3	34.4	34.7
10	25.3	24.2	31.1	30.5
Ranges of standard errors	.19 to .21	.15 to .18	.53 to .59	.36 to .46

[a] Average milk production for a test day multiplied by 30.5 estimates monthly production. The sum of the ten monthly values estimates milk production for 305 days.

for 2 × and 3 × for the same age appear parallel, with the 3 × production larger than 2 × production. Since these curves are parallel, the same factors might be useful for extending 2 × and 3 × lactations; i.e., perhaps milking frequency is not an important factor to consider.

Single test-day relations. Milk produced each single test day is correlated with the amount produced every other test day for the two milking frequencies and two age groups in Table 2. As expected, adjacent months are correlated more closely than nonadjacent months. In general, these correlations are higher for the "under 3" than the "3 and over" groups, with only small differences between frequencies of milking. The lowest correlations between adjacent months appear between first and second, illustrating the increased variability of production on first test day, due in part to the varying stage of lactation at first test. Since these records were computed on a calender-month basis, it was not possible to ascertain average days in production attributed to first test day. Michigan DHIA–IBM Holstein data (8) averaged 33.5 days for first test period, ranging from 4 to 56 days.

In order to ascertain which single test days are most closely associated with total production, correlations between single test-day milk production and the sum of milk production for the ten test days were computed. These correlations (presented in Table 3) were highest for the 5th mo., but high also for the 4th, 6th, and 7th mo. Since correlations between part and whole are partially automatic, one might expect the highest correlation between that single month which is the largest part of the whole, i.e., the month of maximum production. However, variability must be considered also and the single months 4 to 7 have the lowest variability and, therefore, are correlated more closely with the total production than are the months of maximum production (1st to 3rd mo.). As expected from the data in Table 2, correlations are higher for the "under 3" than for the "3 and over" groups, but are about the same for the two milking frequencies.

TABLE 2

Correlations between single test-day milk production [a] month of test

Month of test	2	3	4	5	6	7	8	9	10	2	3	4	5	6	7	8	9	10
	(2 × — under 3 yr.)									*(3 × — under 3 yr.)*								
1	.79	.72	.68	.64	.62	.58	.54	.49	.38	.76	.67	.64	.59	.60	.53	.50	.47	.39
2		.86	.82	.78	.74	.69	.65	.59	.49		.84	.82	.78	.75	.69	.63	.63	.53
3			.88	.84	.80	.74	.70	.63	.52			.86	.83	.81	.74	.70	.69	.56
4				.89	.85	.80	.75	.68	.56				.89	.84	.78	.73	.73	.61
5					.89	.85	.81	.74	.62					.87	.84	.78	.79	.68
6						.89	.85	.78	.65						.89	.81	.78	.64
7							.89	.80	.68							.86	.83	.70
8								.87	.74								.83	.71
9									.84									.85
	(2 × — 3 yr. and over)									*(3 × — 3 yr. and over)*								
1	.73	.64	.58	.54	.48	.43	.36	.30	.23	.70	.61	.57	.53	.48	.44	.38	.29	.21
2		.82	.76	.71	.63	.55	.48	.41	.33		.82	.77	.69	.62	.57	.50	.41	.32
3			.85	.80	.72	.65	.56	.48	.39			.85	.76	.71	.65	.57	.49	.38
4				.87	.79	.73	.65	.56	.45				.85	.77	.73	.64	.56	.44
5					.87	.80	.73	.64	.51					.85	.80	.71	.65	.52
6						.87	.80	.70	.57						.88	.78	.69	.56
7							.88	.79	.65							.86	.77	.64
8								.87	.73								.85	.72
9									.86									.85

[a] All correlations differ significantly from zero at $P < 0.01$.

Regressions of total production on single test-day production are presented in Table 4 and, as in Table 3, the largest values occurred during the 4th to 7th mo.

Cumulative test-day relations. Since most routine production testing programs provide "total-to-date" production, the data in this study also were considered in a cumulative manner. Correlations between cumulative test-day production and the sum of production for ten test days are presented in Table 5. These correlations become larger as the cumulative part becomes an increasingly larger part of the total production, and in all groups are .9 by the 4th mo. of cumulative production.

To ascertain if different extension factors are required for low- or high-producing cows, quadratic regression equations of total on cumulative part production were fitted for the various age–milking frequency groups. The resulting multiple correlation values were within .006 of the correlation values presented in Table 5. These small differences indicate that the same relationship between part and whole production exists for low as for high producers, as has been noted previously (7).

TABLE 3

Correlations between single test-day milk production and the sum of milk production for the ten test days [a]

Milking frequency	2×		3×	
Age	Under 3 yr.	3 yr. and over	Under 3 yr.	3 yr. and over
Test day				
1	.75	.67	.71	.66
2	.86	.80	.86	.80
3	.89	.86	.89	.85
4	.92	.89	.91	.89
5	.93	.91	.93	.90
6	.93	.90	.92	.89
7	.91	.88	.91	.88
8	.90	.84	.87	.84
9	.85	.78	.88	.78
10	.74	.68	.77	.67

[a] All correlations differ significantly from zero at $P < 0.01$.

TABLE 4

Regression of the sum of milk production for ten test days on single test-day milk production

Milking frequency	2×		3×	
Age	Under 3 yr.	3 yr. and over	Under 3 yr.	3 yr. and over
Test day				
1	6.24	4.84	6.16	4.78
2	6.95	5.74	7.43	5.69
3	7.40	6.40	7.34	6.12
4	7.96	7.06	7.88	7.04
5	8.31	7.66	7.93	7.48
6	8.36	7.80	7.78	7.87
7	8.24	7.81	8.22	7.82
8	8.03	7.58	7.91	7.62
9	7.56	7.12	7.90	7.16
10	6.28	5.84	6.46	5.78

TABLE 5

Correlation between cumulative test-day production and total production[a]

Milking frequency	2 ×		3 ×	
Cumulative months	Under 3 yr.	3 yr. and over	Under 3 yr.	3 yr. and over
1	.75	.67	.71	.66
2	.85	.79	.84	.79
3	.90	.85	.90	.86
4	.93	.90	.93	.90
5	.95	.93	.96	.93
6	.97	.95	.97	.95
7	.98	.97	.98	.97
8	.99	.99	.99	.99
9	1.00	1.00	1.00	1.00

[a] All correlations differ significantly from zero at $P < 0.01$.

After deciding to use two age groups, one must develop appropriate factors for extending incomplete records to a 305-day basis. Some workers (1, 2, 9, 10) have obtained factors by the ratio method, where the ratio of total to cumulative part production provides a factor for extending the part to the total basis. Other workers (6, 7, 9, 10) have used a regression equation, whereby cumulative part production is multiplied by the regression, *(b)*, of whole on part and added to an appropriate constant, *(a)*, to estimate total production.

Factors for extending part production to a 305-day basis are presented in Table 6, where *c* represents the ratio of total to cumulative part production,

TABLE 6

Regression and ratio factors for extending cumulative test-day production to a 305-day basis

Cumulative months	Under 3 yr.			3 yr and over		
	a[a]	*b*[a]	*c*[b]	*a*	*b*	*c*
			2 × Milking frequency			
1	87.68	6.24	8.36	146.46	4.84	7.42
2	38.76	3.68	4.15	72.64	3.06	3.70
3	20.02	2.65	2.82	39.74	2.30	2.53
4	9.40	2.10	2.16	21.19	1.87	1.97
5	2.19	1.76	1.77	9.54	1.60	1.64
6	−1.62	1.52	1.51	0.89	1.41	1.42
7	−4.04	1.34	1.32	−4.62	1.27	1.26
8	−4.27	1.20	1.18	−6.80	1.16	1.14
9	−3.16	1.09	1.08	−5.94	1.08	1.06
			3 × Milking frequency			
1	117.14	6.16	8.69	185.22	4.78	7.89
2	42.06	3.86	4.31	97.41	3.08	3.88
3	18.02	2.78	2.91	62.13	2.29	2.64
4	6.59	2.18	2.22	41.52	1.86	2.03
5	1.79	1.80	1.81	25.27	1.59	1.68
6	2.11	1.53	1.54	12.17	1.41	1.45
7	−2.37	1.35	1.34	5.12	1.27	1.28
8	−4.59	1.21	1.20	−0.06	1.16	1.16
9	−3.39	1.09	1.08	−2.42	1.08	1.07

[a] *b* represents the regression of whole on cumulative part production and *a* represents the appropriate constant in the regression method.

[b] *c* represents the ratio of whole to cumulative part production.

and a and b are appropriate constants for the regression equation. These are utilized as follows:

(a) Ratio method: The ratio factor multiplied by total-to-date production estimates 305-day production. From Table 1, cumulative 4 mo. 2×—under 3 yr. production would be (41.3 + 42.0 + 39.3 + 37.1) times 30.5, or 4,871 lb. This, multiplied by 2.16 (from Table 6) would be the estimated 305-day production (10,521), which must conform to the sum of all values in Column 1 of Table 1, multiplied by 30.5, since this is an average situation.

(b) Regression method: Using the same production data as above, but a and b values from Table 6, total-to-date production (4,871) multiplied by 2.10 and added to 30.5, times 9.40, equals 10,516 lb.

Regressions of total on cumulative part production (Table 6) were tested for differences between milking frequencies within age groups. The regressions for 1st mo. for young cows differed significantly. No other comparisons differed significantly, indicating that milking frequency is not of major importance in extending part production.

The ratio method may underestimate total production of low-producing cows and overestimate total production of high-producing cows (10), since the ratio method corrects only for the incompleteness of the lactation and does not take into account the incomplete repeatability of the parts of the lactation which is included in the regression method. The amounts of this difference and of the increased variance of the difference between estimated and actual total production have been presented by Harvey (7). The difference is $(b - c)\ (X - \bar{x})$, where b represents the linear regression of total on cumulative part, c represents the ratio of total to part, and X and $\bar{x}$ represent cumulative and mean cumulative part production. Empirically (7, 10), and as reported previously (10), this difference $(b\text{-}c)$ is negative and largest $(c > b)$ during the early (1-3) cumulative months, least during the center (4–6) $(c \geqq b)$, and positive and small during the longer (7-9) cumulative parts. The increased variance of the difference between estimated and actual total production becomes $(b - c)^2\ \sigma\ x^2$, where $\sigma\ x^2$ equals the variance of cumulative part production.

The choice between methods (ratio versus regression) depends on the purpose for which the method is to be used and the ease of use. The ratio method may appeal intuitively to many and is easier to develop and use. The variation in the total production estimated by ratio is more nearly like the variation in actual total production. In addition to the adjustment for incompleteness accomplished by the ratio method, the regression method adjusts also for unidentified sources of variation which make the part larger or smaller than average, and the total estimated by regression varies less than the actual total. As noted above, the differences between estimates by the two methods are largest during the early months.

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RELATIONSHIPS BETWEEN PLASMA PROTEIN-BOUND IODINE AND CERTAIN MEASURES OF REPRODUCTIVE AND LACTATIONAL PERFORMANCE IN DAIRY CATTLE [1]

H. D. LENNON, JR.,[2] AND J. P. MIXNER
Department of Dairy Industry, New Jersey Agricultural Experiment Station, Sussex

SUMMARY

Plasma protein–bound iodine values (PBI) were determined at 60 days postpartum on 93 Holstein cows (139 lactations) and 34 Guernsey cows (57 lactations) over a 3-yr. period. This measure was correlated on "among lactations" and "among cows in lactation" bases, with certain concurrent measures of reproductive and lactational performance. Measures of reproductive performance included interval from parturition to conception, interval from parturition to first estrus, interval from first breeding to conception, and number of services per conception. Measures of lactational performance included 240-day 4% FCM production, maximum initial 4% FCM yield, persistency index, fat percentage, and solids-not-fat percentage.

The Holstein data on "among lactations" basis gave significant coefficients of correlation between PBI and interval from first breeding to conception (-0.67^{*}) and services per conception (-0.69^{*}). On an "among cows in lactation" basis, the Holstein data also gave significant coefficients of correlation between PBI and interval from parturition to conception (-0.23^{**}), interval from first breeding to conception (-0.25^{**}), services per conception (-0.21^{*}), and fat per cent (-0.18^{*}). No other coefficients of correlation in either breed reached the level of statistical significance.

From the known therapeutic effects of thyroidally active substances on productive processes in various classes of animals, it would be expected that a relationship exists between the level of normal thyroid activity and these productive processes. Kunkel *et al.* (6), in preliminary studies, found significant coefficients of correlation between the level of serum protein–bound iodine (PBI), a measure of blood thyroxine, and the rate of weight gain in beef cattle. Their data suggested that there may be an optimum PBI for maximal growth which if not reached or is exceeded will be associated with lowered rates of weight gain. In subsequent studies by this group (7), variable coefficients of correlation were found between these factors in different trials. In some groups of cattle significant curvilinear coefficients of correlation were obtained.

In trials with swine, Gawienowski *et al.* (3) found a significant negative coefficient of correlation (−.60) between PBI and average daily gain in weight. Sørensen and Moustgaard (16), also working with swine, found a coefficient of correlation of −.45 between thyroxine secretion rate (milligrams per animal per

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[2] Present address: Armour and Company, Research Division, 1425 West 42nd Street, Chicago 9, Illinois.

day) and average daily gain in weight, and a coefficient of correlation of −.63 between thyroxine secretion rate per animal per day per 100 kg. of body weight to the 0.7th power and average daily gain in weight. From these same data, the present authors calculated a coefficient of correlation of .06 between PBI and average daily weight gains.

Sørensen (15), in a study of 99 dairy cows, reported a coefficient of correlation of .33 between PBI and the daily production of butterfat. Also, in a series of 13 cows he reported a coefficient of correlation of .90 between the daily production of butterfat and the logarithm of the thyroxine secretion rate per animal per day per 500 kg. of body weight to the 0.7th power. From data presented on these same animals, the present authors calculated a coefficient of correlation of −.14 between PBI and daily production of butterfat.

Reineke and Soliman (13) have reviewed the role of the thyroid hormone in the reproductive physiology of the female. However, no work has come to the attention of the present authors which would indicate whether or not the activity of the thyroid gland is related to the level of reproductive performance in "normal" dairy cows. It has been shown that thyroidectomized cows failed to show physical signs of estrus (1, 17) but that ovulation and pregnancy occurred (17).

The purpose of this paper is to present the relationships which were found between PBI and certain measures of reproductive and lactational performance in Holstein-Friesian and Guernsey cows. A brief report has been made on this work (9).

EXPERIMENTAL PROCEDURES

Data for this study were collected during the period from 1955 to 1958 on cows in the experimental herd of the Dairy Research Farm, New Jersey Agricultural Experiment Station, Sussex. These included 139 reproduction and lactation records from 93 Holstein cows and 57 reproduction and lactation records from 34 Guernsey cows on twice-a-day milking (10).

Plasma PBI as an index of thyroid activity was determined in duplicate on each animal at 60 days postpartum, using the method of Brown *et al.* (2) as modified by Lennon and Mixner (8).

Measures of reproductive performance for each cow included service interval (days from parturition to conception), the interval in days from parturition to first estrus, the interval in days from first breeding to conception, and the number of services per conception.

Lactational characteristics were represented by 240-day 4% FCM production, maximum initial 4% FCM yield, persistency of lactation, fat percentage, and the per cent of solids-not-fat as previously outlined (10). Test-day data collected once a month were used to calculate the lactation characteristics, in which equal quantities of morning and evening milk on test day were combined and a single Babcock fat test made. Monthly milk yields were calculated on a 4% FCM basis (12) and accumulated to 240 days. Maximum initial milk yield was the highest test-day 4% FCM production. The persistency index as outlined

by Lennon and Mixner (10) was the measure of persistency of lactation. Solids-not-fat (SNF) were performed according to the method of Watson (18) and the per cent SNF were calculated on a 240-day basis. All animals were weighed at 30 days postpartum.

The PBI, lactation, and reproduction records were segregated according to breed and lactation number. The data from each breed were subjected to a least-squares correlation analysis on "among lactations," and "among cows in lactation" bases. The "among lactations" relationships would reflect those due to aging, as indicated by increase in lactation number or body weight; whereas, the "among cows in lactation" relationships would reflect animal differences and would be independent of the effects of age or increasing maturity.

RESULTS AND DISCUSSION

The means of the various breed characteristics as outlined in the experimental procedures are presented in Table 1.

An analysis of variance of the original data indicated that there were no significant "breed" or "lactations in breed" differences in levels of PBI.

The relationships which existed among the four measures of reproductive efficiency for the two breeds of cows are presented in Table 2. The zero-order coefficients of correlation were derived on an "among cows in lactation" basis. In general, the service interval, interval from first breeding to conception, and services per conception were highly correlated to each other; whereas, the interval from parturition to first estrus had rather low correlations with the three other measures.

The relationships which existed among 240-day 4% FCM, maximum initial 4% FCM, and pesistency index in this study have been reported (10). On an "among cows in lactation in breed" basis, both maximum initial 4% FCM and persistency index were positively correlated to 240-day 4% FCM, yet had no relation to each other.

TABLE 1
Means of various characteristics by breeds

Characteristics	Holsteins	Guernseys
No. of animals *(total)*	93	34
No. of records *(total)*	139	57
Lactation *(No.)*	3.24	3.49
Age *(mo.)*	58.6	62.7
Body wt. *(lb.)*	1,267	1,086
PBI *(μg.%)*	4.01	3.85
Reproductive characteristics		
Service interval *(days)*	98.8	107.6
Partum to first estrus *(days)*	54.1	47.6
First breeding to conception *(days)*	24.1	35.6
Services per conception *(No.)*	1.73	2.05
Lactational characteristics		
240-day 4% FCM *(lb.)*	10,034	8,251
Maximum initial 4% FCM *(lb.)*	54.5	45.3
Persistency index *(%)*	89.1	89.2
Fat *(%)*	3.88	4.64
Solids-not-fat *(%)*	8.45	8.98

TABLE 2

Coefficients of correlation among measures of reproductive performance in Holstein and Guernsey cows on an "among cows in lactation" basis

Characteristics	Coefficients of correlation and degrees of freedom	
	Holsteins	Guernseys
Degrees of freedom	129	49
Service interval *versus*		
Partum to first estrus	.17	.39**
First breeding to conception	.84**	.84**
Services per conception	.74**	.62**
Partum to first estrus *versus*		
First breeding to conception	−.10	−.01
Services per conception	−.14	.42**
First breeding to conception *versus*		
Services per conception	.89**	.85**

* and ** indicate statistical significance—$P < .05$ and $P < .01$, respectively.

TABLE 3

Coefficients of correlation between plasma PBI levels at 60 days postpartum and measures of reproductive and lactational performance

Characteristics	Coefficients of correlation and degrees of freedom			
	Among lactations		Among cows in lactation	
	Holstein	Guernsey	Holstein	Guernsey
Degrees of freedom	8	6	129	49
Age *(mo.)*	−.27	.47	.04	−.06
Reproductive characteristics				
Service interval	−.35	.41	−.23**	.07
Partum to first estrus	−.31	.70	.02	.24
First breeding to conception	−.67*	.39	−.25**	−.01
Services per conception	−.69*	.27	−.21*	−.01
Lactational characteristics				
240-day 4% FCM	−.21	.55	.03	.19
Maximum initial 4% FCM	−.13	.58	.01	.04
Persistency index *(%)*	.01	−.17	−.12	−.15
Fat *(%)*	.17	−.57	−.18*	.13
Solids-not-fat *(%)*	.18	−.45	.02	.18

* and ** indicate statistical significance—$P < .05$ and $P < .01$, respectively.

The coefficients of correlation between plasma PBI levels at 60 days postpartum and measures of reproductive and lactational performance are presented in Table 3. In the Holstein data, both on the "among lactations" and "among cows in lactation" basis, there was a tendency for better reproductive performance (shorter service interval, shorter interval from first breeding to conception, and fewer services per conception) to be associated with higher PBI values. No corresponding trend was noted in the Guernsey data. From regression analysis of plasma PBI values associated with measures of reproductive efficiency on an "among cows in lactation" basis in the Holsteins, it was found that for every 1.0 μg. % increase in PBI, there was a decrease in the service interval of 7.8 days, a decrease in the interval from first breeding to conception of 7.4 days, and a decrease of 0.2 services per conception. It is interesting to note that in the female mouse the injection or feeding of thyroidal materials caused an increased mammary gland growth response to estrogen and progesterone (11), an increased

rate of growth (5), increased ovarian and uterine weights (14), and an increased ovarian responsiveness to equine gonadotrophin (4). The present study as it relates to Holstein but not Guernsey cows is suggestive of a positive relationship between thyroid function and reproductive function.

With the exception of the correlation coefficient of −.18* between PBI and fat per cent in the Holstein data on an "among cows in lactation" basis, no other correlations between PBI and measures of lactational performance achieved statistical significance. This study thus fails to confirm the report of Sørensen (15), which showed a positive relationship between thyroid function (as measured either by PBI or by thyroid secretion rate) and lactational performance.

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STUDIES ON THE EXPERIMENTAL PRODUCTION AND PREVENTION OF BLOAT IN CATTLE. III. THE EFFECT OF VEGETABLE OIL AND ANIMAL FAT ON ACUTE LEGUME BLOAT [1]

HARRY W. COLVIN, JR.,[2] J. M. BODA, AND THOMAS WEGNER
Department of Animal Husbandry, University of California, Davis

SUMMARY

The influence of animal and vegetable fats on ruminal motility, eructation, and bloat has been investigated in dairy cattle receiving freshly harvested alfalfa tops under laboratory and feed-lot conditions.

Five eructation and rumen motility trials were conducted on a nonlactating cow having a transected trachea and a ruminal fistula. It was found that the administration of vegetable oil before alfalfa feeding prevented the development of bloat symptoms. Oil administered during the development of bloat increased the rate of eructation and the volume of gas expelled and reduced intrarumen pressure within 15 min.

In the feed-lot trials, eight intact cows, selected for bloat susceptibility, were fed freshly chopped alfalfa with and without the addition of fat. Three types of feeding regimes were followed using reversal type experiments: (1) alfalfa alone; (2) alfalfa containing vegetable oil added at the rate of 1 ml. per pound, and (3) a prefeeding of a concentrate mixture containing various levels of animal tallows followed by alfalfa. The incidence and severity of bloat were estimated with a tympanometer. Fat intakes in excess of 0.06 lb. per head per feeding period prevented bloat for the period immediately following their administration. Bloat prevention was accompanied by a marked and statistically significant increase in alfalfa consumption. These effects did not carry over to the subsequent feeding period, even at intakes of 0.5 lb. of tallow per head.

It is concluded that foaming of the rumen ingesta is an important factor in the etiology of acute legume bloat. Rumen motility studies indicate that there is a sufficient number of secondary (eructation) contractions occurring during the development of bloat to relieve the pressure, if the cardia can be cleared of foam. Animal and vegetable fats prevent acute legume bloat by exerting such an effect.

It is becoming evident that acute legume bloat in ruminants results from an intense frothing of the rumen ingesta (3, 5, 6, 16, 18, 25, 27, 31, 32) accompanied by a fulminating fermentation of the succulent legumes (8, 16). Presumably, the accumulation of foam in the region of the cardia physically prevents eructation (8, 17), although it may prevent eructation by stimulating inhibitory fibers of the eructation reflex (13).

Johns (18), Reid (28, 29, 30), and Reid and Johns (31) have studied the effectiveness of many antifoaming agents in the treatment and prevention of acute bloat in cattle. Other in vivo work with antifoaming agents has been done by Quin *et al.* (26), Momoli-Mucci (24), Dorn (11), Clark (6), Brown *et al* (4), and Erwin and coworkers (14). In vitro experiments concerned with the foam-

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[1] In cooperation with the Animal Disease and Parasite Research Branch, Agricultural Research Service, USDA.

[2] Present Address: Department of Animal Industry and Veterinary Science, University of Arkansas, Fayetteville, Arkansas.

ing properties of various plant and animal materials, foam stabilizers, foam-dispersing agents, and foam inhibitors have been investigated by Lienert and Kienel (21, 22, 23) and by Kolb (20). In vitro experiments with antifrothing agents have also been undertaken by Dougherty and Meredith (13).

The present work was conducted for two purposes: *(1)* to study the effect of a vegetable oil, administered intraruminally before and after feeding freshly cut immature alfalfa tops, on the frequency and volume of eructations and related ruminal phenomena, and *(2)* to study some practical aspects of using vegetable and animal fats to control acute legume bloat.

EXPERIMENTAL PROCEDURE

In the laboratory phase of this work, a mature nonlactating cow of known bloating history was fitted with a rumen fistula and tracheael cannula (10). It was housed in a stall free of bedding, where water was available from a drinking cup.

It was previously established (8) that 15 lb. of freshly cut alfalfa tops could be eaten in a short period of time, and would produce bloat which could be studied under laboratory conditions. In the present work, the cow was fed alfalfa tops for four days before the first eructation trial. At the time of each trial, an average of 14 lb. of alfalfa tops was consumed in an average of 21 min. The total amount of alfalfa tops fed per day was an average of 61 lb.

The laboratory trials were run on consecutive days, using immature alfalfa tops cut fresh daily from the same field. Five trials were conducted in the following sequence: two trials where 175 ml. of Wesson oil (Wesson Oil and Snowdrift Sales Co., New Orleans, Louisiana) were administered 45 min. (70 min. on the figures) after alfalfa tops ingestion; two trials where 175 ml. of Wesson oil were administered intraruminally before alfalfa tops were fed, and, lastly, a trial where no oil was used.

Hereafter, the first two trials (oil administered after bloat had begun to develop), the second two trials (oil administered before alfalfa tops were fed), and the control trial will be referred to as Trials 1, 2, and 3, respectively.

The frequency and volume of eructation were determined by the transected tracheal technique (10). The eructation measuring apparatus was installed 25 min. after the beginning of eating, which would be equivalent to 50 min. on the various figures.

Eructation was measured coincident with rumen motility, which was recorded according to the intraruminal pressure technique (8). After each trial, the character of the ruminal ingesta was noted.

To test the application of the findings of the laboratory studies, experiments under practical feeding conditions were designed. Eight cows, selected for bloat susceptibility, were fed fresh alfalfa tops ad libitum for a 2-hr. period beginning at 8 A.M. and again at 1 P.M. The alfalfa tops were harvested with a field chopper, in the prebloom stage of maturity, at approximately 7 A.M. each morning. At the end of each 2-hr. feeding period, alfalfa consumption was determined for each animal. Thirty minutes after the start of each period and at 15-min.

intervals thereafter, until the intrarumen gas pressure returned to the pre-feeding levels, the degree of bloat was estimated with a tympanometer (19), with the rumen relaxed.

After a four-day preliminary period, the animals had attained a fairly stable alfalfa intake, and moderate-to-severe bloat was observed in most of the animals. They were divided randomly into two groups. At the morning feeding period, one group received fresh alfalfa containing vegetable oil (Wesson oil) added at the rate of 1 ml. per pound of fresh alfalfa. The other group served as controls. Oil was not given at the afternoon feeding period to either group. This procedure was followed to allow the disappearance of any residual effect of the oil by the subsequent morning, and to determine if the effect of the oil on bloat would carry over to the afternoon period. The following morning, the two groups were reversed, the second group receiving oil, the first group serving as controls. This reversal procedure continued for four days.

In other trials, two types of animal fat were substituted for Wesson oil. The types of fat used were commercial yellow grease with a titer of approximately 38° C., and fancy bleachable tallow with a titer of 41.5° C. The saponification value of both tallows was 196. The experimental plan was similar to that outlined above in evaluating the effect of vegetable oil, except that the tallows were incorporated into a concentrate mixture consisting of nine parts ground barley and one part dried beet pulp. The concentrate mixtures were fed approximately 15 min. preceding the morning alfalfa-feeding period. Control animals received an equivalent amount of concentrates. Concentrates were not given at the afternoon feeding period to either group. The concentrate intake per animal varied from 2 to 5 lb.; the amount of tallow added to the concentrate, from 2 to 10%. Reversal-type trials were conducted at each level of concentrates and added tallow. The number of morning observations at each level varied from nine to 28. Control observations were equivalent in number.

RESULTS

The effect of administering Wesson oil before and after feeding alfalfa tops, on intraruminal pressure, is shown in Figure 1. When the oil was introduced before feeding, the pressure was subatmospheric for the duration of the trials. In the absence of the vegetable oil, intraruminal pressure increased rapidly and exceeded atmospheric for the entire trial. However, if oil was placed into the rumen after bloat had begun to develop, there was a rapid reduction of intrarumen pressure within 15 min.

The frequency of eructation was normal (8) during Trial 2 (oil administered before feeding, Figure 2). Within 15–20 min. (85–90 min. in Figure 2) after the introduction of oil into the rumen of the bloated animal (Trial 1), eructation rate became equivalent to that of Trial 2. Thereafter, eructation rates were essentially equal, 16–18 per 30 min. In the absence of oil (Trial 3), the frequency of eructation did not attain this level until 140 min. (115 min. after feeding). It then progressively decreased and the cow remained bloated.

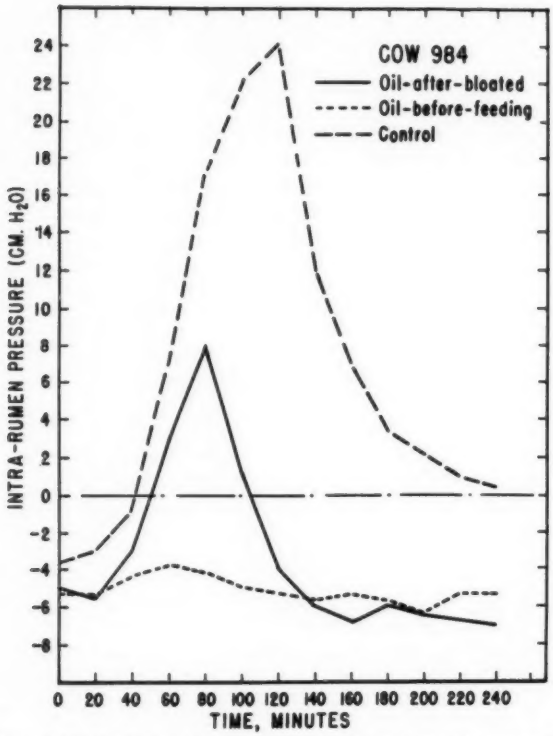

FIG. 1. Effect of administering 175 ml. of a vegetable oil into the rumen before and after feeding alfalfa tops, on intrarumen pressure. In the trials where the cow was permitted to bloat before it was treated, the vegetable oil was administered at 70 min.

The volume of gas eructated per 30-min. period as a result of the various treatments is shown in Figure 3. Within 40 min. (110 min. in Figure 3) after the oil administration in Trial 1, there was a release of over 40 liters of rumen gas. By 170 min., the volumes of gas eructated per 30-min. period of the various oil trials were approximately equal. Practically all of the gas evolved as a result of rapid fermentation of the alfalfa tops was retained in the rumen until 110 min. (85 min. after feeding) in Trial 3. The volume of gas eructated per 30-min. period in Trial 3 did not equal that initially observed in Trial 2 until 140 min. The volumes eructated per 30-min. period on all treatments were approximately equal at 260 min., although the volume in Trial 3 appeared to be dropping off faster than the others. Once again, this is reflected in the maintained positive intraruminal pressure (Figure 1).

The rates of intraruminal fermentation of the freshly cut immature alfalfa tops were approximately equal for the different treatments (Figure 4). The gas within the rumen was eructated almost as rapidly as it was evolved in Trial 2. This did not take place until 130 min. in Trial 1. Apparently, this point was

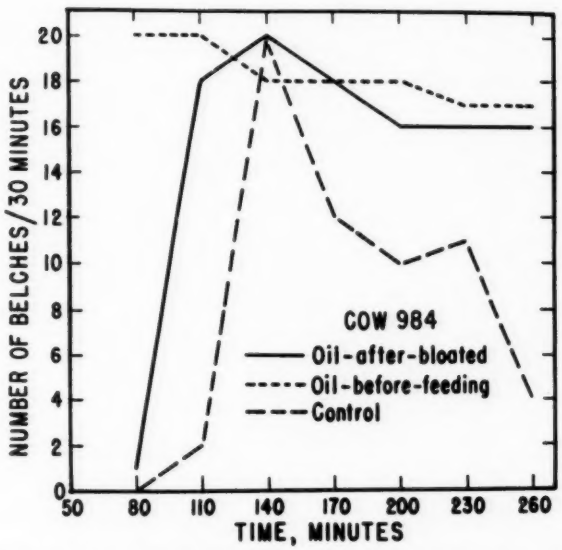

FIG. 2. Effect of administering 175 ml. of a vegetable oil into the rumen before and after the feeding of alfalfa tops, on the number of belches per 30-min. period. In the trials where the cow was permitted to bloat before it was treated, the vegetable oil was administered at 70 min.

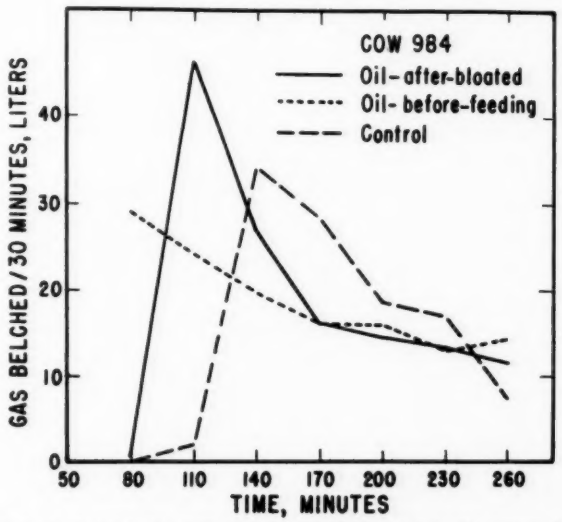

FIG. 3. Effect of administering 175 ml. of a vegetable oil into the rumen before and after the feeding of alfalfa tops, on the volume of gas belched per 30-min. period. In the trials where the cow was permitted to bloat before it was treated, the vegetable oil was administered at 70 min.

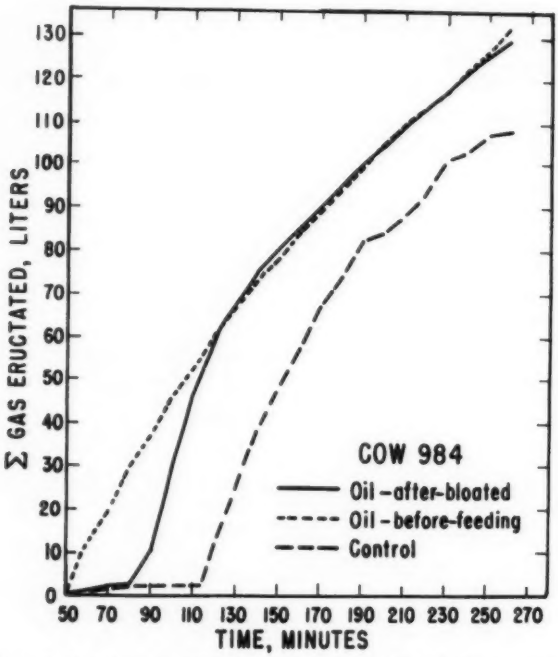

FIG. 4. Effect of administering 175 ml. of a vegetable oil into the rumen before and after the feeding of alfalfa tops, on the sum of gas eructated at progressive intervals following feeding. In the trials where the cow was permitted to bloat before it was treated, the vegetable oil was administered at 70 min.

never reached in Trial 3, as indicated by a difference of approximately 30 liters at 270 min. between the oil-treated and control trials.

An analysis of the rumen motility records revealed the close correlation ($r = .95$) between intraruminal pressure and the ratio of secondary (eructation) contractions to primary contractions, previously referred to by Colvin and coworkers (8). A comparison of the curves representing Trial 3, in Figures 1 and 5, shows that the frequency of the secondary contractions associated with eructation was greatest when the intraruminal pressure was at its maximum. Oil given after bloating had begun reduced the frequency of the secondary contractions to that occurring when oil was introduced into the rumen before feeding. There appeared to be a time lapse in the former.

The average total number of eructations and secondary ruminal contractions were 123 and 128, respectively, in Trial 2. In Trial 1, the average total number of eructations and secondary contractions were 104 and 133, respectively. If the number of eructations and secondary contractions occurring before the administration of the oil are disregarded, the average total number of eructations and secondary contractions in Trial 1 were 95 and 97, respectively. When no oil was used, there were 58 eructations and 213 secondary contractions.

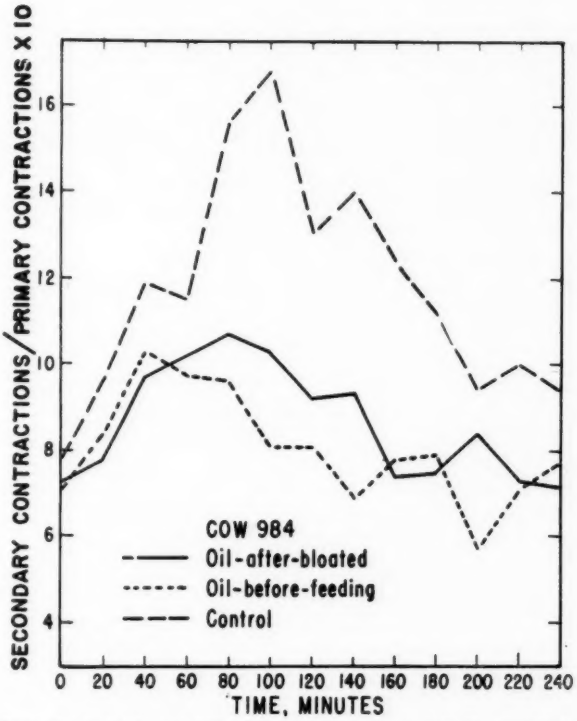

FIG. 5. Effect of administering 175 ml. of a vegetable oil into the rumen before and after the feeding of alfalfa tops, on the ratio of secondary rumen contractions to primary. In the trials where the cow was permitted to bloat before it was treated, the vegetable oil was administered at 70 min.

In the corral-feeding experiments, it was found that the addition of oil to the alfalfa at the morning feeding period prevented bloat at that period (Table 1). The average maximal intrarumen pressure attained in the cattle receiving oil (16 observations) was approximately 1 mm. Hg above atmospheric pressure (range 0–7 mm. Hg). In the control cattle (24 observations), the average pressure was 27 mm. Hg, with a range of 7–50 mm. Hg above atmospheric pressure. The difference between the maximum intrarumen pressures in the control and test groups of cattle was significant at the 1% level. In one case, when the intrarumen pressure reached 50 mm. Hg, the animal was treated effectively by introducing (via stomach tube) 100 ml. of Wesson oil into the rumen.

The incidence of bloat in the presence and absence of oil is shown in Table 4. Since each experimental group contained four animals, it would be possible to observe a maximum of four cases of bloat per feeding, or eight cases per day. The total possible number of bloat cases, for example, in the control group of the vegetable oil experiments was 44. When no oil was applied to the alfalfa

TABLE 1

Maximal intrarumen pressures observed in dairy cattle fed freshly harvested alfalfa tops with and without the addition of vegetable oil[a] at the morning feeding period

Date	Without added vegetable oil					With added vegetable oil				
	Group No.	Fresh alfalfa consumption		Maximum intrarumen pressure		Group No.	Fresh alfalfa consumption		Maximum intrarumen pressure	
		Mean	Range	Mean	Range		Mean	Range	Mean	Range
		(lb.)		*(mm. Hg)*			*(lb.)*		*(mm. Hg)*	
Morning feeding period										
7-31-56	I[b]	44	38–51	37	27–50					
7-31-56	II[b]	47	36–51	30	23–40					
8- 1-56	II	45	38–49	26	10–38	I	48	45–49	0	.....
8- 2-56	I	48	45–49	18	7–33	II	46	42–49	0	.....
8- 3-56	II	45	34–49	24	16–34	I	49	47–50	2	0–7
8- 4-56	I	45	40–48	27	11–42	II	48	45–49	2	0–7
Mean values		46		27			48		1[c]	
Afternoon feeding period										
7-31-56	I	48	45–49	12	6–20					
7-31-56	II	52	45–63	15	9–22					
8- 1-56	II	48	46–49	16	12–21	I	48	48–49	12	9–14
8- 2-56	I	48	48–49	10	7–13	II	48	46–49	12	9–15
8- 3-56	II	49	47–50	15	11–26	I	50	49–50	10	7–13
Mean values		49		14			49		11	

[a] 1 ml. oil (Wesson) per pound of fresh alfalfa.
[b] Four animals per group.
[c] Statistical significance at 1% level.

before it was fed, some degree of bloat was observed in all animals in each period. This was true for all the animals, since the groups were reversed every other day. Without the oil treatment, 26% of the bloat cases were mild in nature, 45% moderate, and 29% severe. Applying Wesson oil to the freshly cut alfalfa reduced the bloat incidence to 11% of the total observations. All of these cases of bloat were of the mild type.

There appeared to be little residual effect of the oil in preventing bloat at the afternoon feeding period, although bloat in all animals was much less severe in the afternoon. The average pressure attained in the animals which had received oil in the morning was 11 mm. Hg (7–15 mm. Hg), as compared to 14 mm. Hg (6–26 mm. Hg) for the controls.

Tallow intakes exceeding 0.06 lb. per animal completely prevented bloat at the morning feeding period (Tables 2 and 4). With the yellow grease, a few mild cases of bloat were observed in animals receiving 0.06 lb., and one case of severe and several cases of moderate bloat in those with an intake of only 0.04 lb. (Table 3).

Statistically highly significant increases of alfalfa consumption at the morning feeding periods were associated with the prevention of bloat by fancy tallow (Table 2). The average alfalfa consumption by the tallow-fed animals (80 observations) was 39 lb., compared with 27 lb. for the controls (80 observations). The average alfalfa consumption by the animals receiving yellow grease at levels greater than 0.04 lb. per head was 39 lb. (65 observations), com-

TABLE 2

Maximal intrarumen pressures observed in dairy cattle fed freshly harvested alfalfa tops after receiving concentrates or concentrates containing fancy animal tallow[a] at the morning feeding period

Observations	Concentrate intake	Without added tallow				With added tallow			
		Fresh alfalfa consumption		Maximum intrarumen pressure		Added tallow	Fresh alfalfa consumption		Maximum intrarumen pressure	
		Mean	Range	Mean	Range		Mean	Range	Mean	Range
(No.)	*(lb.)*	*(lb.)*		*(mm. Hg)*		*(%)*	*(lb.)*		*(mm. Hg)*	
Morning feeding period										
24	2	26	17–43	14	0–26	3	35	27–49	1	0–11
16	2	26	19–49	19	0–34	5	42	28–55	0	.....
16	2	30	16–48	22	6–36	10	42	29–50	0	.....
24	5	25	8–39	19	0–31	10	38	4–49	0	.....
Mean values		27		18			39[b]		0[b]	
Afternoon feeding period										
24	0	32	18–46	12	0–28	....	32	21–48	9	0–29
16	0	35	25–46	17	6–28	....	34	22–39	15	6–22
16	0	29	19–39	11	0–20	....	35	16–46	11	0–28
24	0	28	14–48	12	0–31	....	32	12–46	6	0–33
Mean values		31		13			33		10[c]	

[a] Commercial bleachable fancy tallow.
[b, c] Statistical significance at 1 and 5% levels, respectively.

pared with 34 lb. by the controls, a difference which was statistically significant at the 5% level (Table 3).

The effect of the fancy tallow and yellow grease on bloat, even at intakes of 0.5 lb. per head, did not carry over to the afternoon feeding period to any appreciable extent. The average afternoon intrarumen pressures of the animals which had received fancy tallow at the morning feeding period was 10 mm. Hg (0–33 mm. Hg); the comparable value for the controls was 13 mm. Hg (0–31 mm. Hg) (Table 2). Even though the difference between these means was statistically significant (5% level), there were a number of moderate cases of bloat in both groups and it is doubtful if the difference is of practical importance. Both the grease-fed and control groups had average intrarumen pressures of 12 mm. Hg (Table 3).

DISCUSSION

Acute legume bloat observed under field conditions has been treated with antifoaming agents with varying degrees of success (1, 18, 26). The effect of animal fats and vegetable oils on reducing the intrarumen pressure of cattle induced to bloat on legumes under laboratory conditions has been reported by Reid and Johns (31) and by Boda (2). The present data confirm these results. In the treatment of acute legume bloat, the antifoaming agents presumably function so as to disperse the frothing ingesta, thus simultaneously releasing the entrapped gas and clearing the cardiac orifice. Within 10–15 min. after the oil was administered to the bloated cow in the present work, the eructation rate was equivalent to that of the same animal in the unbloated state (Figure 2).

The rumen motility data indicate that the secondary contractions of the

TABLE 3

Maximal intrarumen pressures observed in dairy cattle fed freshly harvested alfalfa tops after receiving concentrates or concentrates containing crude animal tallow[a] at the morning feeding period

Observations	Concentrate intake	Without added tallow					With added tallow			
		Fresh alfalfa consumption		Maximum intrarumen pressure		Added tallow	Fresh alfalfa consumption		Maximum intrarumen pressure	
		Mean	Range	Mean	Range		Mean	Range	Mean	Range
(No.)	*(lb.)*	*(lb.)*		*(mm. Hg)*		*(%)*	*(lb.)*		*(mm. Hg)*	
Morning feeding period										
16	2	....	.....	....	.....	2	36	12–48	11	0–36
16	2	26	18–40	19	0–40	3	33	6–50	4	0–13
12	5	38	14–59	8	0–25	3	41	22–49	0	.....
9	2	40	20–48	4	0–15	5	44	39–49	0	.....
28	2	30	19–50	12	0–40	5	39	22–49	0	.....
Mean values		34		11			39[c]		1[b]	
Afternoon feeding period										
16	0	26	12–40	15	0–30	....	28	19–44	14	0–33
12	0	38	20–47	11	0–23	....	34	17–45	10	6–14
9	0	39	22–47	10	6–20	....	40	32–48	12	8–14
24	0	32	19–44	12	0–30	....	29	12–39	10	0–31
Mean values		34		12			33		12	

[a] Commercial yellow grease.
[b, c] Statistical significance at 1 and 5% levels, respectively.

rumen associated with eructation were greater in number during the elevated intrarumen pressure than when the intrarumen pressure was normal (Figure 5). In fact, there was a positive correlation ($r = .95$) between the frequency of the secondary contractions and intraruminal pressure. Therefore, it appears that eructation in the bloated animal is impeded by the presence of the froth in the region of the cardia, rather than for strictly physiological reasons.

Recently, Dougherty and coworkers (12) have presented evidence to indicate

TABLE 4

Incidence and severity of bloat in three experiments with cows fed freshly cut immature alfalfa[a]

Treatment	Incidence of bloat (Per cent of total observations)			
	None	Mild	Moderate	Severe
Alfalfa without vegetable oil	0	25	46	29
Alfalfa with vegetable oil[b]	88	12	0	0
Alfalfa preceded by 2–5 lb. concentrates without animal tallow	11	44	40	4
Alfalfa preceded by 2–5 lb. concentrates with 0.06–0.5 lb animal tallow	96	4	0	0
Alfalfa preceded by 2–5 lb. concentrates without animal yellow grease	26	48	18	8
Alfalfa preceded by 2–5 lb. concentrates with 0.06–0.15 lb. animal yellow grease	88	12	0	0

[a] The eight cows in each trial were divided into control and treated groups in reversal experiments.
[b] One milliliter Wesson oil per pound of fresh alfalfa.

that froth in the cardia might stimulate fibers responsible for the inhibition of eructation. Due to the nature of their animal preparations, and an observed lack of rumen motility throughout the experiments, it is difficult to correlate their findings with acute legume bloat. Such a correlation might have been possible had they been able to show a cessation of the secondary ruminal contractions (eructation contractions) coinciding with the lack of eructation. On the other hand, the cardiac orifice is open only for a short period at the time of each rumen eructation contraction, and the presence of froth in this region could be sufficient in itself to impede the passage of any free gas.

The corral-feeding data demonstrate that the use of animal and vegetable fats will effectively prevent acute legume bloat in cattle, at least for a limited period of time after administration. Presumably, any carry-over effect of the fats from one feeding period to the next is reduced by natural movement of the rumen ingesta to more posterior areas of the digestive tract. Similar results also were obtained by Reid and Johns (31), who examined the effectiveness of many different antifrothing agents.

The effectiveness of the oil in the enhancement of eructation can be seen in Figure 2. When the alfalfa tops diet was not supplemented with oil, the eructation frequency was essentially zero until 110 min. By this time, 58 eructations had occurred in the trials where oil was administered before feeding. A similar lag in eructation also took place in the trials where oil was not administered until 70 min. Within 15 min. the eructation frequency was equivalent to that observed where the oil was administered before feeding. Thus, it is apparent that if foam can be prevented, the animal is capable of voiding the gases produced during the rapid fermentation of the succulent legumes. The possible mode of action of the various types of antifoaming agents used in bloat treatment and control has been discussed by Reid and Johns (31).

It has been suggested that grass hays exert their bloat-protecting action by preventing the formation of froth (7, 9). In this regard, it is of interest to note the close agreement between the eructation frequency curves of animals on an alfalfa tops–oat hay diet (8) and the cow being fed alfalfa tops following treatment with Wesson oil (Figure 2). On an alfalfa tops diet, eructation was impeded for over an hour following eating, in both series of experiments. If either hay or oil was included in the diet, eructation was regular and sufficiently rapid to prevent large accumulations of gas in the rumen. Since the evidence is very much in favor of oils preventing bloat by exerting antifrothing action, and since the curves of eructation frequency are remarkably similar for the effect of oat hay and oil, it seems reasonable to assume a similar mechanism of action, at least in part, for both bloat preventives, i.e., oil and oat hay.

The marked increase of alfalfa consumption associated with the prevention of bloat by fats (Tables 2 and 3) is of obvious practical importance. In their bloat studies with red clover, Johns (18) and Reid and Johns (31) also found that bloat depressed intake. If the red clover plants or animals were treated with antifoaming materials, intake was increased to that of nonbloat-provoking plants.

In management operations where freshly harvested legumes are fed, and where the fats can be added directly to the fresh material or be fed with supplemented concentrates, these findings have direct application. However, because of the brief residual effect of the fats in preventing bloat (a period of less than 4 hr. after administration) their use under pasture conditions is limited, unless methods are available for feeding the fats periodically throughout the day. In this respect, Reid (28) has successfully used a method for spraying oil emulsions on bloat-provoking pastures, and Brown *et al.* (4) have reduced bloat severity by placing a water-dispersible lard oil in the drinking water.

The observations on the nature of the ruminal ingesta in these studies support the theory of foaming as being one of the most important factors in the etiology of acute legume bloat (17, 25, 27, 32), as opposed to other theories suggested by Huffman and Cole (15) in the recent NAS–NRC revised bloat review. If oil was not used, the ingesta in the fistulated cow were so copious in amount that they flowed freely from the rumen fistula whenever the plug was removed. The administration of oil before feeding, or after bloating had begun to develop, produced ruminal ingesta which could be sampled only by reaching through the fistula down into the rumen. Instead of the ingesta being light-green and frothy, they were a soupy, dark-green shapeless mass, almost completely devoid of bubbles. Thus, it appears clear that fats prevent the formation of froth, or disperse it after formation, and permit the normal elimination of the rumen gases by eructation.

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OBSERVATIONS ON THE USE OF CHROMIC OXIDE FOR ESTIMATING THE FECAL OUTPUT OF DAIRY ANIMALS [1]

W. A. HARDISON, W. N. LINKOUS,[2] R. W. ENGEL, AND G. C. GRAF

Departments of Dairy Science and of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg

SUMMARY

Two trials employing dairy heifers fed either fresh-cut alfalfa (Trial 1) or mixed hay (Trial 2), and one trial using lactating cows which received a complete ration, have been conducted to study further the usefulness of chromic oxide for predicting fecal output. Total fecal collections were made during each trial. In addition, partial fecal samples were obtained at 6 A.M., 12 NOON, 6 P.M., and 12 MIDNIGHT on each day of each collection period. The results indicate that periods of from three to seven days may be required for the indicator to reach a stable level in the feces. The mean recovery rates of chromic oxide from totally collected feces and from the combined 6 A.M. and 6 P.M. samples for Trials 1, 2, and 3, respectively, were 89.4 and 89.7%; 97.5 and 96.4%; 90.0 and 89.7%. Average daily fecal DM outgo was estimated from the chromic oxide concentration of the combined 6 A.M. and 6 P.M. samples, with an error of about 12%. Only in Trial 2 were satisfactory estimates obtained.

Although chromic oxide can not be employed in measuring the digestibility of herbage by the grazing animal, it does have a high potential value in the estimation of fecal outgo, which is a prerequisite to the measurement of dry matter intake. Coup (2) and Hardison and Reid (5) found that satisfactory estimates of fecal production may be effected from the concentration of chromic oxide in a composite sample representing the total production of feces during periods of from four to 14 days. As a result of experiments conducted with hand-fed and grazing cattle (5, 6), it was found that the combining of feces obtained at 6 A.M. and 4 P.M. during periods of five or more days provided samples, of which the chromic oxide concentration allowed accurate estimates of the total fecal outgo to be made. Subsequent experiments by Brannon *et al.* (1) and Smith and Reid (7) supported these earlier findings.

As part of an over-all project on the utilization of forage crops by ruminant animals, several studies concerned with the development of improved techniques and the evaluation of present short-cut methods for estimating intake and digestibility have been conducted at the Virginia Station. The observations reported in this paper were made during the course of such studies.

EXPERIMENTAL PROCEDURE

Three trials were conducted employing both Holstein heifers and lactating Holstein cows. In all trials, chromic oxide was administered twice daily (approximately 7 A.M. and 4 P.M.) in capsule form. One-half of the total daily dose was given morning and evening. Feces were totally collected in each trial.

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[1] Contributing to Regional Project S12.

[2] Deceased.

In addition, partial fecal samples were obtained from each animal at 6 A.M., 12 NOON, 6 P.M., and 12 MIDNIGHT, on each day of the collection period. To check on the disappearance of chromic oxide from the feces, fecal samples were obtained morning and night for two days after total collection had ceased during Trial 1, and for three days after the end of the total collections during Trial 3.

Trial 1. Eight Holstein heifers varying in weight from 350 to 550 lb. were divided into two comparable groups based primarily on body weight. One group was fed the top portion of the alfalfa plant, and the second group, the bottom portion of the plant. The alfalfa was first-cutting and was fed in the fresh, long state. Daily consumption ranged from about 45 to 60 lb. per head.

The trial was divided into a four-day preliminary period and a seven-day collection period. Each animal received a total of 15 g. of chromic oxide daily throughout the trial, with the exception of the last day of the collection period.

Trial 2. Six Holstein heifers weighing from 450 to 660 lb. were used in this trial. The animals were fed an alfalfa-oat hay ration, and daily intake varied from 10.4 to 14.0 lb. The trial was divided into three 18-day periods, with two animals being used each period. Feces were totally collected during the last six days of each period. Chromic oxide administration was started three days prior to the collection of feces and was continued throughout the collection period. Each animal received a daily dose of 3 g.

Trial 3. Nine lactating Holstein cows receiving a ration of mixed hay, grass silage, and concentrates were divided into three groups. One group received chromic oxide at the rate of 5 g. daily. The second group received 15 g. daily, and the third group 45 g. daily. The trial consisted of a three-day preliminary period and a five-day collection period. Chromic oxide was given each day of the trial, with the exception of the last day of the collection period.

RESULTS AND DISCUSSION

Intraday excretion of chromic oxide. The relative recoveries of chromic oxide from feces samples obtained at various times of the day are shown (Table 1). Excretion of the indicator was found to vary significantly with sampling time in all trials. It may be noted that variation in the excretion of this external reference material was greater in the case of the animals receiving top alfalfa than for animals being fed bottom alfalfa. Statistical analysis revealed, however, that the difference in variation between rations was not significant. Size of dose was also found to influence the excretion of the indicator. Excretion was more uniform when a 15-g. daily dose was used than when either a 5-g. or a 45-g. dose was administered. In addition, the variation was greater in the case of the large dose (45 g.) than with the small dose (5 g.). However, statistical analysis of the data showed that size of dose did not significantly affect the excretion pattern of the chromic oxide.

Daily excretion of chromic oxide. The daily excretion patterns of chromic oxide for Trials 1, 2, and 3 are presented in Figures 1, 2, and 3, respectively. In Trial 1, it appeared that a relatively constant excretion rate was obtained within four days after the initial dose of chromic oxide. This is a somewhat

shorter preliminary period than that usually recommended and used (3, 4, 5, 7). However, Lancaster *et al.* (6) obtained good results when a period of only five days was allowed to elapse between initial dosing and first sampling of feces.

TABLE 1
Relative recovery of chromic oxide
(Expressed as per cent of Cr_2O_3 in composite sample)

Sampling time			
6 A.M.	12 NOON	6 P.M.	12 MIDNIGHT
	Trial 1		
92 ± 2.5[a]	103 ± 2.4	104 ± 4.3	117 ± 1.3
100 ± 5.0[b]	97 ± 4.2	106 ± 4.5	97 ± 4.9
	Trial 2		
97 ± 1.4	96 ± 1.4	99 ± 1.9	100 ± 1.4
	Trial 3		
101 ± 3.5[c]	93 ± 2.1	92 ± 1.0	101 ± 2.9
102 ± 3.1[d]	101 ± 3.2	101 ± 3.5	101 ± 5.8
95 ± 2.9[e]	98 ± 2.6	100 ± 4.1	109 ± 2.3

Mean ± S.E. Each figure is the mean of 28, 36, and 15 values for Trials 1, 2, and 3, respectively.
[a] Animals receiving top alfalfa.
[b] Animals receiving bottom alfalfa.
[c] Animals receiving 5 g. Cr_2O_3 daily.
[d] Animals receiving 15 g. Cr_2O_3 daily.
[e] Animals receiving 45 g. Cr_2O_3 daily.

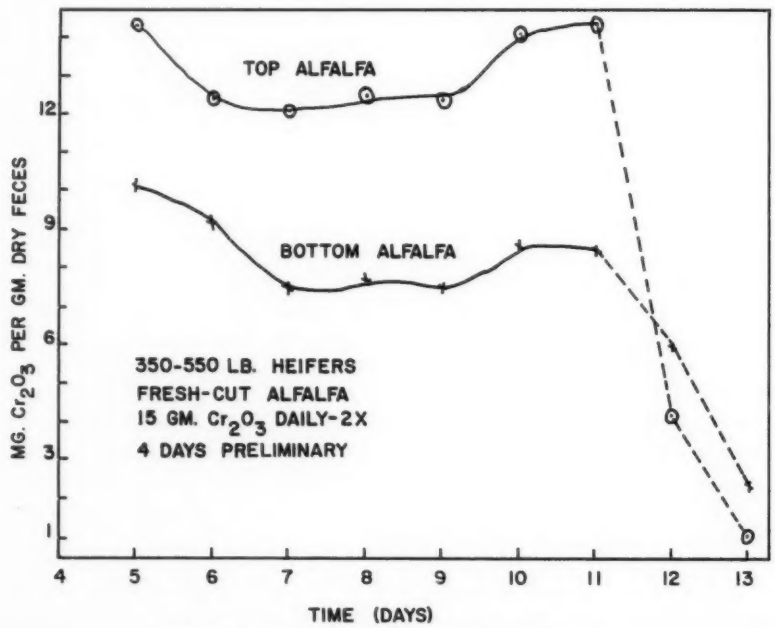

FIG. 1. Daily excretion of chromic oxide (Trial 1).

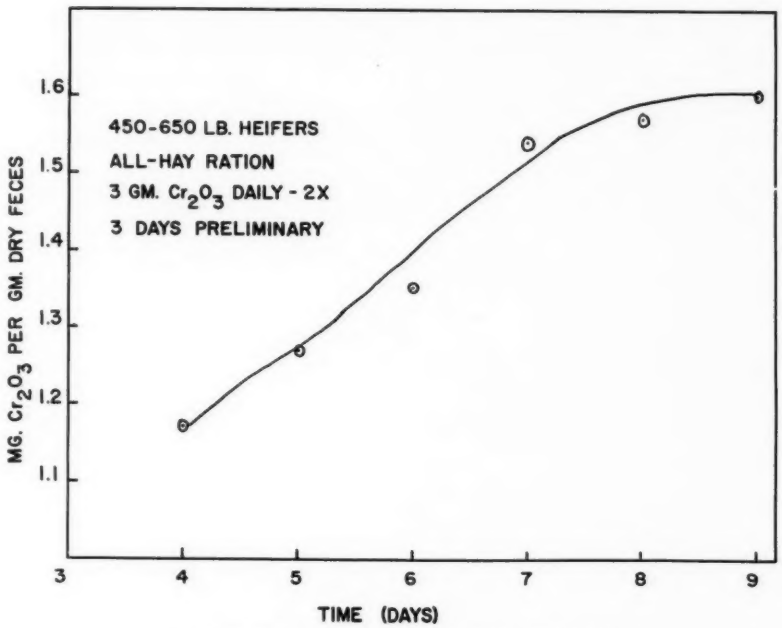

FIG. 2. Daily excretion of chromic oxide (Trial 2).

The significance of the saucer-shaped curves obtained in Trial 1 is not known. Neither is it known why the feces from animals consuming the bottom portion of the alfalfa plant should have a lower concentration of chromic oxide than that of animals consuming the top portion of the plant; however, this simply may be due to animal variation. The higher content of crude fiber in the bottom alfalfa may have contributed to the lower concentration of chromic oxide in the feces, and also to the less steep decay curve obtained with the animals on this particular forage. On the other hand, if it is assumed that rate of passage of the chromic oxide is associated with the crude fiber content of the ration, one might expect to see a stable excretion rate established later with the animals on bottom alfalfa than was the case in this study.

In contrast to the results obtained in Trial 1, the findings of Trial 2 show that a minimum period of seven days was required after initial administration before the concentration of the indicator reached a constant level in the feces (Figure 2). No observations were made in this trial on the excretion of chromic oxide following cessation of dosing.

In view of the excretion pattern obtained in Trial 2, it was felt that perhaps the dose size was too small. Thus, a third trial was conducted in which three different dose sizes were used. The excretion data for this trial are presented (Figure 3). Stable excretion of the reference material was obtained in all cases by the fourth day following the first dose. On a per-unit weight

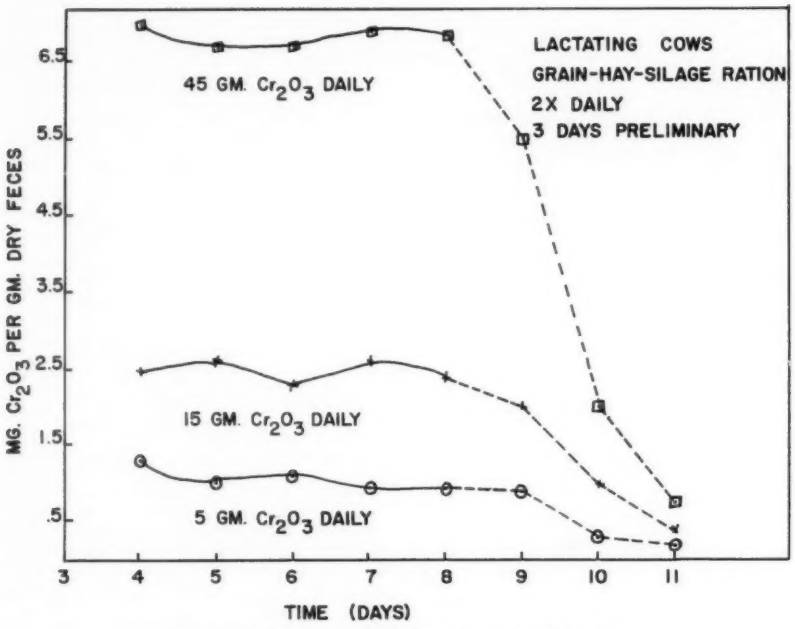

FIG. 3. Daily excretion of chromic oxide (Trial 3).

basis, the 5 g. of chromic oxide given daily in this trial was a smaller dose than the 3 g. given in Trial 2. Therefore, dose size would not appear to explain the relatively long period required in Trial 2 for stabilization of the indicator in the feces.

As shown in Figure 3, the decay of chromic oxide was directly related to the amount of the marker administered.

Estimation of fecal output from grab samples. A study was made of the accuracy with which fecal production could be estimated from the chromic oxide content of combined feces samples taken at 6 A.M. and 6 P.M. throughout each collection period. The results are summarized (Table 2).

In Trial 1, the actual fecal dry matter outgo averaged 2.9 lb. per head daily; the estimated outgo was 3.3 lb. The difference of 0.4 lb. was statistically significant ($P < .01$). In this trial, the mean recoveries of administered chromic oxide from the 6 A.M. and 6 P.M. composite fecal samples and from the totally collected feces were 89.7% (84 to 96%) and 89.4% (81 to 98%), respectively. The recoveries observed on the two types of forage were essentially the same.

Much better recovery rates were obtained in Trial 2: 96.4% (89 to 104%) from the combined 6 A.M. and 6 P.M. samples and 97.5% (89 to 105%) from the totally collected feces. The average estimated fecal output (lb. DM per head daily) in this trial was 4.7 lb., compared to the average actual output of 4.5 lb. This difference was not significant.

TABLE 2
Comparison of measured and estimated outputs of feces

Animal No.	Cr_2O_3 daily	Fecal output	
		Measured[a]	Estimated[b]
	(g.)	*(lb. DM/day)*	
	Trial 1		
1	15	2.2	2.5
2	15	2.1	2.4
3	15	3.2	3.3
4	15	3.7	4.0
5	15	3.3	3.7
6	15	2.6	2.8
7	15	2.7	3.2
8	15	3.6	4.2
	Trial 2		
1	3	4.4	4.9
2	3	4.2	4.3
3	3	4.0	4.5
4	3	4.3	4.3
5	3	5.3	5.1
6	3	4.8	4.9
	Trial 3		
3	5	11.3	12.5
4	5	15.0	16.7
9	5	8.3	6.8
1	15	12.1	13.7
6	15	13.1	13.4
7	15	9.7	12.7
2	45	12.8	16.4
5	45	15.7	18.3
8	45	9.3	11.9

[a] As determined by actual collection.
[b] Based upon concentration of Cr_2O_3 of combined feces samples collected at 6 A.M. and 6 P.M., over periods of from five to seven days.

The worst recovery rates and estimates were obtained in Trial 3, especially with the larger doses of chromic oxide. For the 5-g. dose the recoveries were 101.0% (90 to 123%) and 104.3% (92 to 125%), from the combined grab samples and total collection, respectively. In the case of the 15-g. dose, the corresponding values were 87.4% (76 to 98%) and 82.9% (79 to 86%), and for the 45-g. dose, they were 80.7% (76 to 86%) and 82.7% (81 to 86%). The errors in estimating fecal outgo from the combined 6 A.M.–6 P.M. samples varied from approximately 4.5% when the 5-g. dose was given, to 26% when 45 g. chromic oxide were administered daily. The average estimated fecal outgo (lb. DM per head per day) was significantly greater than the actual fecal production ($P < .05$).

The reason or reasons for the low recovery of chromic oxide obtained in Trials 1 and 3 are not apparent. All of the possible sources of error were checked.

These data serve to illustrate some of the practical as well as basic problems which may be encountered in studies involving the use of chromic oxide to estimate fecal outgo or dry matter digestibility with ruminant animals. It would appear that the indiscriminate use of the indicator in nutritional studies could result in considerable error.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Juel Albert for his assistance during the conduct of these trials.

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SUPPLEMENTATION OF COTTONSEED HULLS WITH AN ALCOHOL-UREA PREPARATION[1]

E. R. MORRIS[2] AND O. H. HORTON
Department of Animal Industry, Arkansas Agricultural Experimental Station, Fayetteville

SUMMARY

Twenty Holstein heifers were used in a 120-day feeding trial to study the feeding value of a liquid protein supplement, composed of 10% urea, 80% molasses, 6% ethyl alcohol, 4% phosphoric acid, and an assortment of trace minerals, upon growth and feed consumption. Pasture clippings supplemented with 2 lb. of ground corn were fed to six of the heifers; whereas, cottonseed hulls plus 2 lb. of cottonseed meal and 1 lb. of corn were fed to a second group of seven heifers, and cottonseed hulls, 3 lb. of an alcohol-urea preparation, and 1 lb. of corn were fed to a third group of seven heifers. The average daily gains and pounds of feed per pound of gain were 1.21 and 16.91, 0.78 and 29.48, and 0.88 and 29.22 lb. for the groups receiving pasture clippings, cottonseed hulls plus cottonseed meal, and cottonseed hulls plus the alcohol-urea preparation and corn, respectively. There was no significant difference between the two groups of heifers receiving cottonseed hulls in gains per day or gain per pound of feed eaten. The alcohol-urea preparation represented over 80% of the total protein equivalent.

In the feeding of low quality roughages, proper supplementation must be accomplished for optimum results. Previous workers (3, 5, 6, 8, 9, 15, 21, 22) have shown that urea and other nonprotein-nitrogen–containing compounds may replace a portion of the protein in the ration of ruminant animals. King *et al.* (12) compared cottonseed meal and molasses containing 5% urea and ammoniated molasses. The urea and ammoniated molasses group failed to gain as rapidly as did the cottonseed meal group. Parham and associates (14) fed ammoniated molasses and urea which supplied 30% of the protein equivalent in the concentrate, or 18% in the entire ration, and obtained gains of 1.04, 0.92, and 1.23 lb. for ammoniated molasses, urea, and cottonseed meal, respectively. Bohman *et al.* (4) observed that molasses was not an adequate carbohydrate for the utilization of urea for rumen microorganisms fed with little or no starchy carbohydrates and low quality grass hay. The Georgia station work with heifers, reviewed by Reid (16), showed that urea-fed heifers gained 1.4 lb. per day when urea supplied approximately 70% of the total protein equivalent. Kerr (11) studied ammonia nitrogen release and protein synthesis by rumen microorganisms in a Type M Morea containing 6% ethyl alcohol and a Type A Morea containing no ethyl alcohol. Morea with ethyl alcohol excelled in protein synthesis at the end of 12 hr. Lassiter *et al.* (13) fed rations containing 3.0, 5.0, and 7.0% urea to heifers and obtained gains of 1.44, 0.85, and 0.64 lb. per day, respectively.

This study dealt with an evaluation of a liquid alcohol-urea preparation as a

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[1] Approved for publication by the Director of the University of Arkansas Agricultural Experiment Station.

[2] The data in this paper are from a thesis submitted by the senior author in partial fulfillment of the requirements for the degree of Master of Science.

supplement to poor quality roughage for growing heifers. As analyzed by its manufacturer, it was found to be composed of 10% urea, 80% molasses, 6.0% ethyl alcohol, 4.0% phosphoric acid, and an assortment of trace minerals. Pasture clippings and cottonseed hulls plus cottonseed meal were used to compare with the feeding value of this alcohol-urea preparation.

EXPERIMENTAL METHODS

Twenty Holstein heifers from the University of Arkansas dairy herd selected on the basis of body weight and age were divided into three groups. The number of animals in each group, 1, 2, and 3, were 6, 7, and 7, respectively. Allotment into each group was made by matching three heifers near the same age, breed, and weight, and one heifer was then placed in each group by random selection. Each animal was fed individually in tie stalls daily at 4 P.M. The animals remained tied overnight and were turned out at 8 A.M. in a dry exercise lot, where water and a mineral mix were available. The ration for Group One consisted of pasture clippings and 2 lb. of corn; for Group Two, cottonseed hulls, 2 lb. of cottonseed meal, and 1 lb of corn; for Group Three, cottonseed hulls, 1 lb. of corn, and 3 lb. of an alcohol-urea mixture. Rations for each group were formulated so that the nutrient intake on the three rations would be approximately equal. The amount fed to each animal was expected to produce 1.2 lb. of gain per day based on the N.R.C. requirements. The animals were weighed weekly and on three consecutive days each 4 wk. A 21-day preliminary feeding period, to accustom the heifers to the feed and surroundings, was used.

Samples of the roughage and supplements fed were taken each week. After grinding, samples were analyzed using the methods approved by the Association of Official Agricultural Chemists (2). The chemical composition of the rations is shown (Table 1).

An analysis of variance was made on the data for daily gains according to methods of Snedecor (19). The apparent digestibility of the rations fed was determined by using two 600-lb. dairy steers in metabolism stalls similar to

TABLE 1

Chemical composition of ration ingredients

Composition	Pasture clippings	Cottonseed hulls	Cottonseed meal	Corn	Alcohol-urea preparation
Dry matter	90.89	88.29	90.45	88.25	65.33
Crude protein[a]	9.18	3.81	35.58	9.55	31.25[b]
Ether extract	1.36	1.26	3.31	3.07	.....
Crude fiber[a]	33.30	46.58	9.45	2.96	.....
Ash[a]	6.00	2.35	5.93	3.33	10.11
Nitrogen-free extract[a]	40.96	34.39	43.28	69.34	52.04

[a] Per cent calculated on dry matter basis.

[b] Contains nonprotein nitrogen to make protein equivalent content 47.84% on dry matter basis, or 31.25% protein equivalent on wet basis.

Note: The pasture clippings fed in this experiment were the native grasses common to northwest Arkansas. The cutting was made in late June from excess pasture and was known to contain Cheat, Bermuda, Brome, Hop Clover, Fescue, Orchard Grass, and possibly other native grasses.

those described by Horn *et al.* (10). Fecal collections were obtained over a seven-day period in each of three trials.

RESULTS AND DISCUSSION

Data showing the effects of the different rations on body weight changes and feed consumption per pound of gain are presented (Table 2). Group One, receiving pasture clippings, gained 51 and 39 lb. more, respectively, than Group Two, which received cottonseed hulls plus cottonseed meal, and Group Three, which received cottonseed hulls plus an alcohol-urea preparation. Since the difference required for significance between treatments was 37 lb., these differences were considered real differences (Snedecor, 5th edition, section 10.6). The difference between Groups Two and Three was only 12 lb.; thus, it was considered within the limits of experimental error.

In this trial, no visible toxic effects were observed that could be attributed to the alcohol-urea mixture for the duration of the feeding trial. One heifer in Group Two and one in Group Three seemed to be slow in adjusting to cottonseed hulls, and carried a rough hair coat for about 2 mo.

The results of the single-reversal digestibility study for the rations fed to the heifers in Groups Two and Three, and a single collection period for the ration of Group One, are shown (Table 3). The coefficient of digestibility of dry matter, crude protein, and crude fiber appears to be in favor of the ration with pasture clippings. These differences are similar to those found with prairie hay and cottonseed hulls by Essig and Ray (7). Alcohol-urea preparation and cottonseed hulls seem to have improved slightly more the digestibility coefficient for crude protein, crude fiber, ash, and nitrogen-free extract than did the cottonseed meal and cottonseed hulls. Other workers have reported an increase in digestibility of cottonseed hulls with the addition of molasses. It was observed,

TABLE 2
Result of 120-day feeding trial

	Group One	Group Two	Group Three
Group description	Pasture clippings	Cottonseed hulls plus cottonseed meal	Cottonseed hulls plus alcohol-urea preparation
Number of animals	6	7	7
Initial average weight *(lb.)*	716.7	690.1	703.1
Final average weight *(lb.)*	861.8	783.7	808.9
Total average gain *(lb.)*	145.2	93.6	105.7
Standard deviation—total gain/heifer	19	17	36
Gain per day *(lb.)*	1.21**	0.78	0.88
Gain/100 lb. protein *(lb.)*[a]	39.36	37.59	28.68
Gain/100 lb. feed *(lb.)*[b]	5.91	3.39	3.52

** Significantly greater gain per day.
[a] Calculated on dry matter basis.
[b] Calculated on air-dry basis.

Note: The pasture clippings fed in this experiment were the native grasses common to northwest Arkansas. The cutting was made in late June from excess pasture and was known to contain Cheat, Bermuda, Brome, Hop Clover, Fescue, Orchard Grass, and possibly other native grasses.

TABLE 3
Summary of results of digestion study

Steer		Apparent digestibility of nutrients in per cent					
		D.M.	C.P.	E.E.	C.F.	Ash	NFE
Trial 1							
A	Cottonseed hulls plus alcohol-urea	43.9	38.1	27.6	41.4	37.6	76.6
B	Cottonseed hulls, cottonseed meal	36.20	16.6	39.4	30.9	16.8	70.7
	Difference in favor of alcohol-urea	7.7	21.5		10.4	20.8	6.0
	Not in favor			11.8			
Trial 2							
A	Cottonseed hulls, cottonseed meal	42.3	35.2	39.6	40.2	31.6	74.4
B	Cottonseed hulls plus alcohol-urea	44.1	44.5	35.4	37.5	39.5	76.9
	Difference in favor of alcohol-urea	1.7	9.4			7.9	2.49
	Not in favor			4.2	2.6		
Trial 3							
A	Pasture clippings plus corn	55.9	47.2	16.8	52.0	35.3	73.2
B	Pasture clippings plus corn	56.2	50.2	8.4	54.1	25.8	71.9

however, that this alcohol-urea preparation failed to make up for all the difference in quality between cottonseed hulls and pasture clippings, even though the protein equivalent was approximately the same.

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DETERMINATION OF VOLATILE FATTY ACIDS IN BOVINE BLOOD BY ISOTOPE DILUTION [1, 2]

J. B. HOLTER, S. LAKSHMANAN, AND J. C. SHAW
Dairy Department, University of Maryland, College Park

SUMMARY

The levels of acetate, butyrate, and valerate in venous cow blood were determined by an isotope dilution technique involving an acidified acetone-ethanol extraction procedure. It was found that acetate accounts for most of the total volatile fatty acids (VFA) in venous bovine blood and probably is present in greater amounts than heretofore reported. The isotope dilution technique is a valuable tool for the determination of the level of the VFA in blood, but maximum precision and accuracy probably will not be possible until the mode of transport of VFA in blood is understood.

The importance of the volatile fatty acids (VFA) in ruminant nutrition is widely recognized and the subject has been reviewed (7, 20, 21). The level of these VFA in blood reported by different workers varies considerably, perhaps as a result of the use of different extraction techniques. Duclaux (6), Friedeman (9), McClendon (16), and McAnally (14) reported distillation procedures for the removal of the VFA from blood; however, separation of individual acids was not put on a practical basis until the introduction of partition chromatography by Martin and Synge (13). Subsequently, reliable column partition chromatographic techniques have been reported by Elsden (8), Moyle, Baldwin, and Scarisbrick (19), Keeney (12), and Wiseman and Irvin (25), and have been used (15) for the resolution of blood acids; but quantitative recovery of the VFA from blood is still dependent upon the efficiency of extraction.

Annison (1) found 3.3–7.9 mg/100 ml. (mg. %) total VFA calculated as acetate in the venous blood of the cow 2–4 hr. post-feeding. Similar results have been found with the venous blood of the goat (1, 5). McClymont (17) reported 8–12 mg. % of acetate in the arterial blood of cows 2–4 hr. after feeding. The concentration of the VFA (calculated as acetate) in the portal blood of sheep lies in the range of 5–22 mg. % (2, 3, 4, 22, 23, 24). Acetate accounts for over 90% of the VFA concentration in the peripheral blood of the cow (1, 17, 18).

This study was undertaken to establish the upper limits of the acetate, butyrate, and valerate levels in venous cow blood.

METHODS

The blood used in this experiment was drawn 2–3.5 hr. after feeding from a lactating Holstein cow on a normal herd ration. All blood samples were heparinized immediately upon drawing and analysis begun within 10–20 min. Most samples were run in duplicate.

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[2] Data presented in this paper are taken from a thesis submitted by J. B. Holter in partial fulfillment of the requirements for the M.S. degree.

The procedure used to determine acetate was as follows: To 50 ml. of freshly drawn blood was added 72 to 199 μM of acetate-1-C^{14}-Na having a specific activity (SA) of 348 to 4,200 counts per minute (CPM)/μM. After thorough mixing, the two were allowed to equilibrate for 10 min. (except where equilibration time was being studied as a variable), then immediately injected into a mixture of 10 ml. of 50% sulfuric acid (v/v), 400 ml. of ethanol, and 400 ml. of acetone, with vigorous stirring. The precipitate was removed by filtering through Whatman No. 1 filter paper and the filtrate was distilled until one liter of distillate was collected. This distillate was made alkaline with potassium hydroxide and evaporated to dryness. The potassium salts of the VFA were then acidified with 50% sulfuric acid and the acids resolved by the chromatographic method described by Kenney (12). Each fraction, after titration, was evaporated to dryness on a hot plate under a current of air. The residue of potassium salts was made up to volume in water and an aliquot was assayed for radioactivity. The reduction in SA was used to calculate the concentration of acetate in the blood used.

As part of an extraction technique comparison study not reported herein, Sample 1A (Table 1) was first deproteinized with cadmium sulfate according to the method of Fujita and Iwatake (10) and the filtrate treated as above.

The concentration of butyrate in blood was determined by use of the procedure described for acetate with the following modifications: (1) 100 ml. of blood was used; (2) 0.47 μM of *n*-butyrate-1-C^{14}-Na (SA = 200 CPM/μM) was equilibrated with the blood for 10 min.; (3) the blood precipitate was not removed before steam distillation; (4) separation of the acids was accomplished by the chromatographic column developed by Wiseman and Irvin (25), as modified by McCarthy (15).

Blood levels of valerate were determined by the procedure used for butyrate, except that 57.2 μM of *n*-valerate-1-C^{14}-Na (SA = 800–900 CPM/μM) was used.

To provide a check on the results obtained for the extraction, blank deter-

TABLE 1

Concentration of acetate found in freshly drawn cow blood as determined by isotope dilution

Sample No.	Time after feeding	Equilibration time	Acetate
	(min.)		*(mg. %)*
1A[a, b]	145	10	9.6
1B[a]	145	10	9.8
2	210	10	18.7
3A	195	10	18.0
3B	195	30	23.8
4	185	50	22.6
5A	205	5	11.1
5B	205	60	13.9
6A	175	10	17.2
6B	175	15	18.5
7A	180	10	17.3
7B	180	30	13.2
7C	180	60	18.2
7D	180	120	11.9

[a] Acids separated by method of Wiseman and Irvin (25).
[b] Proteins precipitated by method of Fujita and Iwatake (10).

minations for acetate were made, using 50 ml. of water instead of blood, and this was again repeated, using redistilled ethanol and acetone in the method.

The radioactivity in the VFA fractions being studied was measured in the following manner: Each fraction was evaporated to dryness under a current of air. Care was taken to keep the sample alkaline to phenolphthalein during evaporation. This dry residue was then diluted to a known volume with water containing a trace of Triton X-100. Aliquots of this solution were spread evenly on counting planchets, dried, and counted in a continuous gas-flow Geiger counter system. Counting samples were prepared to count at a rate $<7{,}000$ CPM, counting planchets were made up in quadruplicate, and counting was carried out for at least 10,000 total counts.

RESULTS AND DISCUSSION

An amount of labeled acid approximately equal to the concentration anticipated in blood was equilibrated with blood, followed by extraction with an acidified acetone-ethanol mixture and, later, steam-distilled.

The possibility that the solvents might be a source of acetate, either as a natural contaminant and/or as a result of oxidation during steam distillation was investigated. Blank extractions, using the isotope dilution technique on 50 ml. of distilled water, showed 2.4 mg. % of acetate to be present. When both the acetone and ethanol were redistilled over alkali, then used as above, the amount of acetate found was 1.5 mg. %. Appropriate corrections for blank values were made in Table 1.

The acetate concentrations appear to be somewhat higher than those reported earlier for bovine venous blood (1), sheep venous blood (3, 23), and goat blood (1, 5). Day-to-day variations in acetate levels are apparent. Equilibration time seems to have some effect upon the concentration of acetate found, but no simple trend is obvious.

It must be borne in mind that little is known about the transport mechanism of the lower fatty acids in blood. Apparently, there exists some acetate-binding mechanism in blood which first must be elucidated before a successful attempt can be made at determining the true level of acetate. Consequently, at the present time, and with the large volume of blood necessary for identifying individual acids, any extraction method must be somewhat arbitrary.

It will be seen from Table 2 that the concentration of butyrate in the venous

TABLE 2

Concentration of butyrate in freshly drawn cow blood as determined by isotope dilution

Sample No.	Time after feeding	Equilibration time	Butyrate
	(min.)		*(mg. %)*
1A	155	10	0.41
1B	155	10	0.46
2A	200	10	0.66
2B	200	10	0.53
3	120	10	0.53
4	190	10	0.84

TABLE 3
Concentration of valerate found in freshly drawn cow blood as determined by isotope dilution

Sample No.	Time after feeding	Equilibration time	Valerate
	(min.)		*(mg. %)*
1A	170	10	0.00
1B	170	10	0.41

blood of the cow was found to be quite low and of questionable significance. These values are not inconsistent with others reported thus far in the literature, and are in agreement with recent observations in this laboratory (15), that butyrate is normally completely removed from the portal blood by the liver of the goat.

The results in Table 3 indicate that essentially no valerate is present in the venous blood of the cow.

The concentration of VFA in bovine blood observed by this isotope dilution technique is somewhat higher, but proportionally similar, to that reported by McClymont (17, 18).

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TECHNICAL NOTES

MAINTENANCE OF ACTIVE LACTIC CULTURES BY FREEZING AS AN ALTERNATIVE TO DAILY TRANSFER

Daily propagation of lactic cultures involves considerable expenditure of time, media, and materials, and requires careful technique to avoid contamination during repeated transfers. Furthermore, the desirable balance of strains generally present in a freshly combined culture frequently is lost after several propagations. The preservation of an active culture in its optimum condition for flavor and acid production has been attempted by Johns (3), Olson (5), and Heineman (1). This report deals with the technique studied by the writers in a similar attempt to maintain lactic cultures at optimum activity without daily transfers.

Cultures were prepared by inoculating a sterile milk medium at the rate of 1% with freshly ripened active starter. The inoculated milk was placed at once in an ice cream–hardening room at −20° F. After storage for up to 6 mo., the previously inoculated milk was thawed in about 2 hr. in a water bath at 70° F., then incubated for 16 hr. at 70° F.

Table 1 shows that Culture B, frozen in this manner and tested at monthly intervals, maintained practically the same activity during storage for 6 mo. Cultures from four commercial sources were tested similarly and responded equally well after storage at −20° F.

Table 2 emphasizes the need for freezing the culture medium soon after inoculation, as incubation for more than 6 hr. before freezing resulted in a less active culture after storage. Varying the level of inoculum from 1 to 10% did not influence activity. Most modifications of milk media normally used for culture maintenance were suitable for the frozen cultures. Fresh fluid skimmilk plus 2% dry skimmilk appeared slightly superior in these trials. The cultures were frozen in 16-oz., wide-mouthed, Nalgene HH plastic containers.[1] This container was used repeatedly for sterilizing milk at 15 lb. steam pressure for 15 min., without severely damaging the container. Freezing, thawing, and subsequent incubation were performed in the same plastic container.

Cultures prepared as described above were supplied to a local commercial dairy, where they were used to inoculate bulk starter. Buttermilk was made successfully five times weekly over a period of 3 mo., using a frozen culture to inoculate each bulk starter. Frozen cultures also were used in this manner for processing buttermilk and cottage cheese in the Clemson College Dairy. The activity of the frozen cultures compared favorably with fresh starters transferred six days per week.

Preliminary trials indicated that several types of paper milk cartons were suitable for the freezing, thawing, and incubation steps. In these trials, commercially pasteurized and cartoned milk was used. Since serial transfers are eliminated in this system, it is felt that pasteurized rather than sterilized milk can be used. By inoculating carefully pasteurized milk before filling the cartons, it should be practicable to prepare at one time enough cultures for 3–6 mo. of operation.

J. C. Simmons [2]
D. M. Graham [3]
South Carolina Agricultural Experiment Station, Clemson

[1] Made by the Nalge Company, Inc., Rochester, New York.

[2] Dairy Industry Supply Association Fellow.

[3] Present address: Pet Milk Company, Research Laboratory, Greenville, Illinois.

TABLE 1

Culture B tested at monthly intervals during storage at −20° F.

Storage time	Developed acidity	pH	Leber's test (4)	Elliker's Test (2)	Creatine Test[a]	Aroma
(mo.)	*(%)*		*(min.)*	*(% acidity)*		
0	0.90	4.50	30	0.47	++	Good
1	0.60	4.45	30	0.34	+++	Good
2	0.75	4.60	30	0.41	+++	Good
3	0.77	4.50	30	0.36	+	Good
4	0.82	4.50	30	0.41	+	Good
5	0.85	4.50	30	0.36	+	Good
6	0.78	4.55	30	0.38	+	Good

[a] +++ indicates intense, ++ moderate, + slight, and − absence of red color.

TABLE 2

Culture A incubated for different periods of time before and after storage at −20° F.

Incubation time (hr.)[a]								
Before freezing	After freezing	Total time	Developed acidity	pH	Leber's Test	Elliker's Test	Creatine Test[b]	Aroma
			(%)		(min.)	(% acidity)		
0	16	16	0.88	4.60	30	0.44	++	Excellent
2	14	16	0.89	4.65	30	0.39	++	Excellent
4	12	16	1.17	4.65	30	0.45	+	Good
6	10	16	1.16	4.50	30	0.40	++	Good
8	8	16	0.64	4.95	45	0.30	−	Fair
10	6	16	0.59	4.90	60	0.25	+	Fair
12	4	16	0.89	4.65	45	0.29	++	Fair
14	2	16	0.91	4.60	60	0.29	++	Fair
16	0	16	0.90	4.70	45	0.30	++	Excellent

[a] After thawing the cultures which were incubated less than 16 hr. before freezing, incubation was resumed for the interval necessary to give a total incubation time of 16 hr.
[b] See footnote [a], Table 1.

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ABSORPTION OF COLOSTRUM GLOBULINS INTRODUCED DIRECTLY INTO THE DUODENUM

It has long been recognized that there is intestinal absorption of immune globulins from the colostrum by the newborn calf for a limited time after birth. Mason *et al.* (4) suggested that the intestinal absorption of globulins by the newborn may be the incapacity for digestion, or that the gut of the newborn is more permeable at birth than later, or both.

Hill (2) suggested that the cessation of intestinal absorption of immune proteins from colostrum by the newborn of several species coincides with the development of gastric protein digestion. One explanation of this relationship is that after gastric digestion is initiated colostrum proteins are digested. Colostrum contains an antitryptic factor that normally may protect the proteins from pancreatic proteases (3). Deutsch and Smith (2) fed an aluminum-hydroxide gel and probanthine to a calf for 32 hr. postpartum, to inhibit gastric activity. A test globulin was fed at 40 hr., but there was no absorption of the globulin. However, no test was made to determine whether there was diminished gastric activity.

This study was undertaken to ascertain the intestinal permeability to immune proteins from colostrum introduced directly into the duodenum of calves varying in age.

Holstein calves were deprived of colostrum and maintained on mature milk prior to treatment. Age difference served as the experimental variable. The calves were anesthetized with sodium pentabarbital and the intestines exposed. The duodenum was ligated approximately 3 in. posterior to the pyloric sphincter.

About 1.5 liters of colostrum were introduced into the intestine posterior to the ligature via a polyethylene tube. Then the duodenum was ligated posterior to the point where colostrum was introduced, and the incision was closed by suturing the peritoneum, muscles, and skin. Blood was withdrawn from all calves prior to and at 4, 8, and 12 hr. following the introduction of colostrum. All serum samples (0.01 ml.) were subjected to duplicate electrophoretic fractionations in Spinco Model R B electrophoresis apparatus on paper strips in sodium veronal buffer (pH 8.6, $\eta = 0.075$) for 16 hr. The ages of the various calves at the time of infusion were: (A) 6 hr., (B) 18 hr., (C) 48–60 hr., (D) 48–60 hr., and (E) 48–60 hr. The operation and colostrum infusion of Calf E was performed without anesthesia.

Electrophoretic patterns of the blood serum proteins before and after the introduction of colostrum into the duodenum of Calves A and E are shown on the left and right, respectively, of Figure 1. Serum protein patterns of Calves

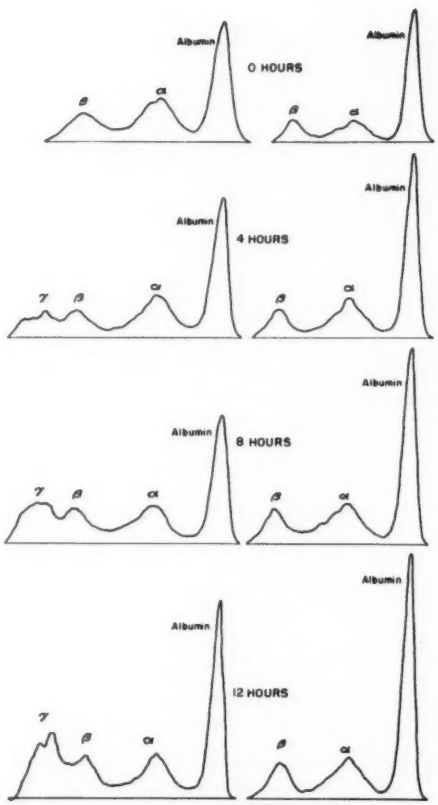

FIG. 1. Electrophoretic patterns of the blood serum proteins before and at 4, 8, and 12 hr. after the introduction of colostrum into the duodenum of Calf A (left) and Calf E (right).

A and B were similar, whereas Calf E typified C and D. The serum protein patterns for Calves A and B (6 and 18 hr. old, respectively) exhibited increasing amounts of gamma globulins 4, 8, and 12 hr. following colostrum administration. However, older calves (C, D, and E) did not absorb the immune proteins from the colostrum that was introduced into their intestines. Because the intestinal tracts were flooded with massive amounts of colostrum, the lack of gamma globulin appearance in the serum of the older calves indicates that gastrointestinal enzyme development is not the primary reason for the inability of older calves to absorb immune proteins per se. The results of the study presented here suggest that the permeability of the intestinal wall of the young calf to colostrum globulins is transitory.

VEARL R. SMITH
E. S. ERWIN
Departments of Dairy and of Animal Science,
University of Arizona, Tucson

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COMPARISON OF GLASS AMPULES AND PLASTIC VIALS BASED ON THE NONRETURN RATE OF FROZEN BOVINE SPERMATOZOA

Many different types of containers have been used for the storage of frozen bovine spermatozoa. Few data have been reported on the effect plastic vials or glass ampules may have on the fertility of frozen semen. VanDemark and Kinney (9) found no significant difference in the freezability of five semen samples frozen in glass ampules or plastic vials. Brugman and Schmidt (1) reported on a limited number of animals bred and found that fertility was not altered by type of container. Dunn *et al.* (2) reported on experiments conducted on a split-sample technique to compare the fertility of frozen semen stored at −79° C. in hermetically sealed glass ampules and in polyethylene bulbs. A total of 298 inseminations were made with semen frozen in glass ampules, and 313 inseminations were made with semen frozen in the polyethylene bulbs. The 60- to 90-day nonreturn rates were 60.7 and 62.3%, respectively. The difference of 1.6% in favor of polyethylene bulbs as containers for frozen semen was not significant. However, Musgrave and Heath (7) reported a highly significant difference in percentage of motile spermatozoa between glass ampules and plastic vials, in favor of the glass ampules.

A series of three trials has been conducted by the authors. In the first trial, semen was collected on a split-sample basis from five bulls. The semen was frozen in a yolk-citrate extender, using the techniques as outlined in References 3, 4, 5, and 6. Samples were used over a 60-day period.

TABLE 1

Comparison of glass ampules with plastic vials based on the nonreturn rate of frozen bovine spermatozoa

Glass ampules			Plastic vials		
First services	N.R.	N.R. 75 days	First services	N.R.	N.R. 75 days
(No.)	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*
177	133	75.1	276	174	63.0

The results in comparing glass ampules and plastic vials are shown (Table 1). The difference of 12.1% was highly significant at the 1% level of probability as analyzed by X^2 (8).

The second trial was conducted in an attempt to determine the effect of freezing semen in glass ampules and plastic vials and the effect of low-temperature liquid and dry storage based on nonreturn rate. Semen was collected from 12 bulls, two at each of six different artificial breeding organizations. The semen was extended in yolk-citrate extender and the same techniques utilized in the freezing process as described for the first trial. An extension rate of 1:40 was employed. One-half of the extended glycerolated, equilibrated semen was placed in glass ampules and the other half in plastic polyethylene vials. Following freezing, one-half of each bull's semen frozen in glass ampules and plastic vials was maintained at −79° C. with dry ice and methanol (liquid storage). The other half was maintained at −76° C. with dry ice only (dry storage). The four variables tested were as follows: glass ampules–liquid storage, glass ampules–dry storage, plastic vials–liquid storage, and plastic vials–dry storage. All the semen was stored for 4 wk. before usage, to permit the possible effect of container and method of storage. Thirty-three technicians received three ampules of frozen semen of each of the four variables once each week for 6 wk. The ampules were thawed in a 5° C. water bath and used immediately on first-service cows.

The results of this trial are presented (Table 2). As analyzed by X^2, a significant difference in nonreturn rate existed between glass ampule and plastic vials, irrespective of the method of storage. The difference in nonreturn rate between glass ampule–liquid storage and plastic vial–dry storage was significant at the 1% level. The differences between all other variables were not significant. Nine of the 12 bulls had the highest nonreturn rate when their semen was frozen in glass ampules, irrespective of storage method. Six of 12 bulls had the highest nonreturn rate when their semen was frozen in glass ampules and stored in liquid. There was essentially no difference among the variables in the average estimated progressively motile spermatozoa, as determined by microscopic examination.

The third trial involved the use of a new type polyethylene plastic vial. The semen was extended in a yolk-citrate medium and the same techniques in the freezing process were employed as previously stated. A dilution rate of 1:50 was used. Semen was collected from four different bulls, utilizing both first and second ejaculates on a split-sample basis. Semen was used over a 4-wk. storage period and all samples were stored, submersed under alcohol and dry-ice. Samples were maintained at −79° C. until used, and were thawed immediately before use in a 5° C. water bath.

The results of this trial are presented in Table 3.

TABLE 2

Nonreturn rate of frozen bovine spermatozoa utilizing different types of container and methods of storage

Variable	First services	N.R.	N.R. 75 days
	(No.)	*(No.)*	*(%)*
Glass ampule–liquid storage	434	284	65.4
Glass ampule–dry storage	473	289	61.1
Plastic vial–liquid storage	487	272	55.9
Plastic vial–dry storage	453	255	56.3
Total	1,847	1,100	Average 59.7
Glass ampule (irrespective of method of storage)	907	573	63.2
Plastic ampule (irrespective of method of storage)	940	527	56.1
Liquid storage (irrespective of type of container)	921	556	60.4
Dry storage (irrespective of type of container)	926	544	58.7

TABLE 3

Comparison of glass ampules and the presently marketed plastic vials for frozen semen based on the nonreturn rate of frozen bovine spermatozoa

Glass ampules			Plastic vials		
First service	N.R.	N.R. 75 days	First service	N.R.	N.R. 75 days
(No.)	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*
523	345	66.0	495	305	61.6

The difference of 4.4% in favor of the glass ampule was not significant as analyzed by X^2. However, it is of interest to note that in all three trials the higher percentage of nonreturns occurred with the glass ampule rather than with the plastic vial. For the total of the three trials, 1,607 and 1,711 first-service cows were bred to frozen semen that had been stored in glass and plastic ampules, respectively, with 1,051 and 1,006 nonreturns, respectively. The percentages of nonreturns were 65.4 and 58.9, respectively, for glass ampules and plastic vials.

From these data, the glass ampule for the storage of frozen semen was superior to the plastic vial. Based on improved results with the use of the new vial, it shows much promise and should be equal to glass. Nevertheless, the results with the new plastic vial were lower than with the glass ampule.

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E. F. GRAHAM
W. E. ERICKSON [1]
Department of Dairy Husbandry,
Institute of Agriculture,[2]
University of Minnesota,
St. Paul

[1] Present address: Tri-State Breeders' Cooperative, Westby, Wisconsin.

[2] Paper No. 3918 Scientific Journal Series, Minnesota Agricultural Experiment Station.

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A COMPARISON OF THE ORANGE G DYE AND KJELDAHL METHODS FOR DETERMINING MILK PROTEIN [1]

The orange G dye method as developed by Fraenkel-Conrat and Cooper (2) and modified by Udy (4, 6) for estimating proteins in wheat flour and milk, was compared to the standard macro-Kjeldahl procedure, using a total of 104 samples of whole milk. The dye unbound by the protein was measured in an Evelyn photoelectric colorimeter using a special ab-

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sorption cell (5). The galvanometer readings were converted to protein values by use of a graph. By increasing the time of mixing the sample and dye with a mechanical tumbler from 5 to 10 min., more uniform results on triplicate determinations were obtained.

Milk from Holstein, Jersey, and Guernsey cattle of different ages and in different stages of lactation in two Ohio Agricultural Experiment Station herds was used in this comparison. Samples were collected at three different times approximately 1 mo. apart, but the same cows were not represented each time. The protein values obtained by the standard A.O.A.C. macro-Kjeldahl procedure were compared with values obtained by the orange G dye method. Except for preliminary work of developing facility with the technique, during which triplicate samples were used, all samples have been determined in duplicate for both methods.

The correlation of 0.98 between the results of the two methods (as shown in Table 1) indicate that the orange G method of determining protein in milk is as accurate as the Kjeldahl procedure. In fact, the standard deviations show less variation with the orange G dye than with the Kjeldahl method. Seldom did a sample vary as much as ±0.1 percentage unit from the Kjeldahl value, and approximately two-thirds of the samples fell within ±0.05 percentage unit of the corresponding Kjeldahl value.

TABLE 1

Milk protein valuese determined by orange G method as compared to Kjeldahl Method

	Dye	Kjeldahl
No. of samples[a]	104	104
Mean protein content	3.2475	3.2456
Standard deviation	.0193	.0394
Coefficient of correlation r = .98		

[a] Samples were determined in duplicate for each method.

Ashworth and Seals (1,3) have studied the effect of various factors on dye binding by the different proteins of milk. They have shown that the test may be used under a wide range of temperature conditions, 46–130° F.

Several advantages of the dye method over the standard macro-Kjeldahl procedure are (1) it is from four to five times as fast as the Kjeldahl procedure, (2) the cost per sample is much less, (3) there is no handling of strong acids or alkalies, and (4) it is simple enough to be used routinely.

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J. M. Treece
L. O. Gilmore
N. S. Fechheimer
Department of Dairy Science,
Ohio Agricultural Experiment Station,
Wooster

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A STUDY OF VULVAR FLUORESCENCE IN DAIRY CATTLE

The phenomenon of vulvar fluorescence has been used in human medicine in diagnosing disorders of menstruation and early pregnancy (1–7). The changes in the luminescent colors elicited from the vulva and perineal region by light in the near ultraviolet range (3660 A.) are claimed to reflect changes in ovarian hormone levels under various conditions.

An attempt was made to utilize this technique of vulvar fluorescence in ultraviolet light for evaluating the stage of the estrous cycle in dairy cattle, which would be of particular value in cases of "silent heat."

In this study, a total of 77 observations was made on 36 cows, with some of the cows being observed from three to four times during an estrous cycle. A microscope spot lamp containing a G.E. AH-4 mercury arc bulb was used, with the light passing through 5 mm. of a Corning No. 5860 filter which transmits mainly in the 3660 A. range. The whole lamp

was encased in a box to keep incident light from escaping from the sides and bottom. This lamp gave a spot of light about 5 cm. in diameter when held about 15 cm. from the area of the subject being examined.

All observations were made in total darkness after the eyes had been dark-adapted by wearing red X-ray goggles for at least 5 min. prior to the examination. The vulva and perineal areas of all cows were washed and dried before any observations were made, to eliminate any extraneous fluorescence from accumulated manure and urine. Examination was made of the areas on the inside of the lips of the vulva, around the area of the clitoris, and then of the entire perineum outside the vulva.

For analysis of the data obtained, the cows were grouped according to the stage of estrus, as follows:

I—3–9 days pre-estrus
II—2 days pre-estrus to 4 days post-estrus
III—5–12 days post-estrus
IV—13 or more days post-estrus, more than 12 days pre-estrus
V—Pregnant cows

The intensity of color observed was rated visually on a scale of 1–3, from light to dark.

Very little true fluorescence could be observed on any of the cows examined. A faint green color interspersed with purple varying from faint to dark in intensity was regularly seen on the inside of the labia majora. No significant differences in the intensity of the vulvar fluorescence colors could be demonstrated between the groups of cows at the various stages of the estrous cycle and pregnancy. However, certain trends were noted in the group means. There was a tendency for more intensity of purple coloration during the estrogenic stages of the estrous cycle (Groups I and II). This was not significant and was at least partially attributable to individual differences in skin pigmentation, observational error between days, and possibly some leakage of visible purple through the filter. The intensity of the purple appeared to be related to the skin pigmentation of the perineum, and was darker in black pigmented cows than in white pigmented cows.

The blood vessels of the vulva were plainly visible in the ultraviolet light and showed up as dark pigmented lines. There was considerable variation in the visible vascularity, but no correlation with the presumed hormone levels at the various stages of the estrous cycle was noted.

Two anestrous cows that were treated with intramuscular injections of 4.5 and 9.0 mg. diethylstilbestrol, respectively, demonstrated no changes in the intensity or color of vulvar fluorescence or in vascularity of the vulva, even though both cows came into estrus within 24 hr.

This study did not demonstrate that vulvar fluorescence in U.V. light could be used to accurately reveal estrus in cows ovulating with quiet or silent heat. Some changes and variations were noted, but these were not consistent and could not be correlated with the stage of estrus.

N. L. VanDemark
V. L. Estergreen, Jr.
Department of Dairy Science,
University of Illinois, Urbana

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THE CARBOHYDRATES OF SOME GLYCOPROTEINS OF BOVINE MILK[1]

The presence of carbohydrates in proteins has been recognized for many years. Recent studies have demonstrated that many of the blood and human milk proteins contain appreciable quantities of hexoses, hexosamines, fucose, and/or an interesting amino sugar—"sialic" or "neuraminic acid" (9). Evidence indicating the presence of glycoproteins in milk was furnished first by the work of Storch in 1897 (7), who reported that the fat globule membrane contained a carbohydrate. Recently, Nitschmann (5) characterized a macro-peptide which was released from casein following a rennin treatment. This component, soluble in

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12% TCA, contained 15.2% galactose, 4.3% glucosamine, and 11.4% neuraminic acid (1).

The objective of our studies has been to identify the carbohydrates which are chemically bound to *a*) the soluble protein fraction of the fat globule membrane (4), *b*) the minor protein fraction as reported by Weinstein (8), and *c*) the proteose-peptone fraction (6). Chemical and chromatographic techniques were employed. Protein-bound hexose, hexosamine, fucose, and sialic acid were determined according to the analytical scheme outlined by Glick (3). All of the standards were chemically pure, except for sialic acid which was obtained by using orosomucoid from human blood serum as a secondary standard (11.2% siaile acid). Results of these studies appear in Table 1 and represent average values of three trials in duplicate.

Of interest are the concentration ratios—hexosamine: hexose: fucose: sialic acid. These relationships for the soluble membrane protein, the Weinstein fraction, and the proteose-peptone fraction are 1:2.5:0.65:1.8; 1:3.2:0.44:3.4, and 1:2.5:0.61:1.7, respectively. The high hexose and sialic acid content is characteristic of similar relationship in the blood proteins, an observation which suggests the possible origin of the glycoproteins of milk.

Paper chromatography was used to determine which of the hexoses and hexosamines were present. Twenty-five ml. of solution containing 125 mg. of protein were hydrolyzed for 3 hr. at 100° C. with 2 *N* HCl. Several trials showed these conditions to be optimum for complete liberation of the carbohydrate from the protein. The hydrolysates were dialyzed against three changes of distilled water which were combined, evaporated to dryness, and extracted with pyridine to remove the salts (3). The residue was dissolved in a minimum of 10% isopropanol and spotted on Whatman No. 1 paper. A descending chromatogram was run for 32 hr. at 21°C., using a solvent mixture made up of amyl alcohol, pyridine, and water (4:3:2). The chromatogram was dried at 70° C. for 15 min. and developed at 40° C. in a moist atmosphere with 2% triphenyltetrazolium chloride in an equal volume of 1 *N* NaOH. This developing reagent was superior to either ammoniacal $AgNO_3$ or aniline oxalate. Known carbohydrates were spotted similarly and served as standards in the identification of spots from the protein samples. The results of these chromatograms indicate the presence of fucose, galactosamine (weak), galactose, glucosamine, and mannose in the three protein fractions studied.[2]

TABLE 1

Concentration of carbohydrates in some minor proteins of bovine milk

Carbohydrate	Protein		
	Soluble membrane protein (4)	Weinstein fraction (8)	Proteose-peptone (6)
	(mg/100 mg. protein)		
Hexose	2.69	3.46	2.90
Hexosamine	1.08	1.10	1.15
Fucose	0.71	0.48	0.70
Sialic Acid	2.00	3.60	2.00
Total	6.48	8.64	6.75

M. P. Thompson
AND
J. R. Brunner
Department of Dairy,
Food Technology,
Michigan Agricultural Experiment Station, East Lansing

[2] Sialic acid was destroyed with the above hydrolysis conditions, but was detected chromatographically after 1 hr. of hydrolysis with 2 *N* HCl.

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Symposium. Humanistic and Social Studies in a Technical or Scientific Education[1]

THE LIBERAL ARTS IN SCIENTIFIC-TECHNOLOGICAL CURRICULA

J. W. SHIRLEY
Dean of Faculty, North Carolina State College, Raleigh

The discussion today—Education for What? it might be called—is a very timely one. At no time, at least during my lifetime, have the American people been so aroused about education generally as they are in these post-satellite days. There has been general concern about what we really are doing in our schools and universities; there has been some hysteria, particularly in Washington circles. There has been much talk about science and the intellectual cold war and what must be done to meet these new challenges to our world leadership. These are called days of crisis; there are many prophets crying in the wilderness, though we may be confused as to which prophets we should follow.

We must recognize early, however, that these are not entirely new and different situations we face. Every age has considered itself to be an era of crisis and danger. Every age has faced problems which no previous generation has been called upon to solve. Every age has charged its educational leaders to use the highest quality of imagination and reason in devising ways and means of meeting these problems, both to preserve the traditions of the race and culture and to create an environment which will permit that generation and the ones to follow to grow and flourish in directions which seem desirable to that age. This is the principal function of the conservative, formal organizations of our society—to tie the past, which is known, to the future, which is not known, in terms of the present and its needs.

The special problems which this generation must face are occasioned by the phenomenal growth in the areas of the sciences and technologies, which has given man, for the first time, the means for the destruction of the human race. Although previous generations have been concerned with the direction of man's knowledge to man's welfare, this is the first which has had to face possible annihilation if it does not succeed. This is the underlying factor which has changed the pace of our existence, and brought a sense of urgency to politics, to education, and to every-day life which forces us to action at a new and frantic pace. We no longer have the time nor the inclination to proceed in leisurely fashion. It is this rush of events, this breathless feeling of lack of time, which has engrossed us and made us, as a people and a nation, want to act first and then review our actions after the facts are in. It is this hurried "buy-it-now" attitude which has led some of our legislators to propose educational appropriations for specific ends greater than the total cost of current education. It is this urgency which may lead us to attempt things which are impossible before we start, which makes it imperative that we review our educational patterns with the soundest, most reasoned judgments that we can bring to bear on them.

Like it or not, we must confess that much of the scientific and technological development which has led to our present state and much of our educational confusion of the moment is the result of the Land-Grant College movement. These institutions were founded just over a century ago with the avowed purpose of bringing together the liberal and technological educations which had previously been separate. As one farmer expressed it before the 1855 Michigan General Assembly:

> The day has forever gone by when an enlightened liberal education was deemed useless for a farmer. Agriculture has risen into a science, too, the most comprehensive of all, which demands not alone strong hands and bodily labor, but an active, vigorous, cultivated intellect. . . .

This was normal and right in its day. In America, for the first time, the laboring class was the governing class. Presidents came from log cabins, from farms, and from the traditional professions. Every man needed a trade; yet, at the same time every man was a potential legislator, senator, or ambassador. The democratic ideal of an enlightened citizenry insuring sound government demanded that every man have a universal education.

What was not then realized but is clearly apparent today is that at that moment education was getting itself caught in a future trap. Historically, the idea was sound, and in the light of the state of the educations of the day, the job was not obviously impossible.

[1] Papers presented at the 53rd Annual Meeting of the American Dairy Science Association, North Carolina State College, Raleigh.

Liberal education, scientific education, technological education—all of these were ancient and honorable approaches to the solution of human problems, working from different angles. Liberal education—the education of the free citizen and the governing class in the philosophical and humane pursuits leading to an understanding of man as individuals and as a class—was as old as Plato and the wellspring from which public education, schools, and colleges had evolved. Technical education—the name is allied to the Greek word for "carpenter"—was just as old. This education had nothing to do with the free citizen or the philosophical and moral humanism of the ruling class, since it was designed for the workers or, in Greek and Roman times, for the slaves. Science, too, in that day was a portmanteau term, embracing all knowledge and all learning, though its connotations had changed somewhat since the sixteenth century, to embrace mainly those physical, mathematical, and biological studies which were subject to observational verification. Yet, by present standards, even this science was descriptive and elementary.

What has happened, and this certainly could not have been predicted a century ago, is that all aspects of this education—liberal, scientific, and technological—have, under specialization and expanded activities on all fronts, developed far beyond the dreams or aspirations of the most visionary. Unfortunately, these accretions to knowledge have come gradually and in few cases have negated the previous learning. As a result, in all areas we have piled new knowledge on old, increased the scope and the depth of our courses and our curricula. We smile at the naïveté of Francis Bacon who took all knowledge to be his province, but at the same time we try to force our students to emulate him. We demand that our graduates know—in the same four-year learning period used by past generations—not in general terms, but specifically enough to work problems or to answer true-false questions (usually hastily and poorly devised) on subjects ranging from field theory, Planck's constants, non-Euclidean geometry, set theory, to the ecology of reptiles. At the same time, our educators have reviewed their methods, and have vacillated between pedagogy and subject matter, free electives and required electives, area majors, specializations, concentrations, theoreticals, and applications. Diverse faculty members and departments have reworked the course of study, each intent on including something new, none concerned with taking anything out. Especially in Agriculture, under the terrific impact of the Experiment Stations and their applied and basic researches, have curricula moved in strange and unpredictable ways. Cursory review of college catalogues will reveal that our present-day curricula have generally ceased to be planned courses of study. The word itself seems to have reverted to its original etymological meaning of a racetrack (from the Latin *currere,* to run), or a two-wheeled chariot pulled by two horses.

Yet, at no previous time in American history has it been so important to educate the whole citizen as now. If this is an age of danger, it is a danger occasioned by bad politics rather than by bad science. If we are to preserve those qualities of freedom and independence of mind and soul for which our democracy was dedicated, if we are to survive as a free people in the modern world, we must have a citizenry alert to its opportunities and fit to accept its responsibilities. If we are to capture again the original purposes of the Land-Grant movement, if we are to educate the whole citizen in the light of modern concepts of society, we seriously need to review not only the liberal portion of the curriculum but the scientific and technological portions as well. If we are to produce the balanced individuals we need in a world of crisis, we must ourselves show the balance that we wish to propagate in our students, and must present a possible and balanced four-year program for them that will accomplish the fundamental aims of higher education.

The first thing we must do is to recognize that we can not teach all of science and all of technology in four years, even if we ignore all humane and social content. In both areas we must stop, as quickly as possible, trying to do this impossible. Two fundamental changes in our point of view are needed: (1) We must recognize that the four college years are not terminal in the educational process; they can only put a student into a frame of mind conducive to learning and give him a background against which he can start his life-long education. (2) We must recognize that the first job our students will take should be only a preliminary step in the direction of maturing and greater responsibilities. Considering college as a terminal program is what leads us frantically to include more and more into these four years, to cram the student full of facts and figures, on the off-chance that he may someday need to know them. This is why we have gone from 120 semester hours to 130, to 140, to 150, or to 160. This is why we have force-fed the student with predigested ideas without leaving him the time or the energy to arrive at ideas of his own.

Looking at the student only in terms of his first job, has made us put a premium on the technique, or the gadget, of the moment that it does not deserve. If we know anything at all about machines, techniques, and procedures, it is the fact that they are the most rapidly changing features of our mechanized civilization. Even if it were possible to bring our students up-to-the-minute in these superficialities (ignoring the fact that many faculty members are not up-to-the-minute themselves), we can be certain that by the end of his first five

years on the job, most of our graduate's knowledge will be passé and outmoded.

Back in the days of the depression, a relatively unknown high-school teacher from Manitowoc, Wisconsin, wrote an essay on "The Impossibility of Education" for the debunking *American Mercury*. Mr. Orr contended:

> It is my own personal conviction that degrees and diplomas are entirely meaningless . . . and that a Phi Beta Kappa key is the antithesis of what it is supposed to represent. . . . I charge that any student who achieves superior marks in all his college courses is essentially an extreme conformist who must necessarily accept everything on textbook and pedagogic authority, since he has not the ability to discriminate between the humbug taught by one professor or textbook and the science taught by another.

This is an extreme position, designed to shock. But it does put the finger on the cancer of our present-day educational system—the confusion of ends and means in our over-emphasis on facts and our indifference to students. By our methods and procedures and our curricular practices, we are more concerned with conformity to rules and regulations than with the development of intellect or of taste and discrimination. Perhaps under the pressures of mass education, coupled with indifference to the individual as a human being, we will continue to standardize our product, to check off heads in class attendance, contact hours, C-averages, and paid-up traffic tickets. But we must not be misled into thinking that these things constitute or have anything to do with learning, with wisdom, or with education.

Mr. Orr was not completely negative. He summed up his discussion with three simple objectives which I have often recalled as best stating the real purpose of education, whether liberal, scientific, or technical:

> The characters of true education seem to me to be quite clear and definite. They consist of *(1)* the power to discriminate among relative values; *(2)* a profound appreciation of and reverence for superior values; and *(3)* a fervent spirit of sincere and honest doubt. . . . Any person who possesses the above three requisites is an educated person regardless of age, station, diplomas, or degrees; any person who does not possess *all* the above requisites is *not* an educated person; and it is possible under our modern and popular conception of education to obtain a Ph.D. degree with a Phi Beta Kappa key and not possess a single one of the above requisites.

You will note that in accepting these goals as the objectives of education, no reference has been made to subject matter or to the area of studies involved. Yet, I hold that they constitute the essence of liberal education. They restate the Greek ideal of wisdom coupled with virtue. They bring education down to the individual, human, rational level once more; they make education a process and not an act. They call for analysis, discrimination, appraisal, evaluation, and application of facts and data to human problems rather than as ends in themselves. They call for the educated person to think for himself, to evolve a personally satisfying pattern of values based on a thorough sympathy with an understanding of the values of other men and of other times. They call for an intellectual rebirth as a continuing process, stimulated by a reverence for truth and for the love for man.

If this general view of objectives does not satisfy you (and I am sure it will not satisfy Dr. Gullette, who must bring it down to earth), but you wish to come to grips with the fields of subject matter usually embraced in the liberal arts, let me refer you to the practical objectives arrived at after years of study by a group facing problems similar to your own: the engineers. Stimulated by their earnest desire to make engineering a profession, and being men of action determined to get things done, the engineering schools of the nation established, half a century ago, a Society for the Promotion of Engineering Education (now the American Society for Engineering Education), for the purpose of discussing matters of philosophy and policy in education and of bringing them to operating levels. Like all of us, they were astounded at the terrific advances made in science and technology during World War II, and immediately began a national study to see what impact this accumulation of knowledge would have on their educational procedures. The resultant study, Report of the Committee on Engineering Education After the War (popularly called the Hammond Report after the chairman, Dean Hammond, of Penn State), outlined the three stems of engineering education, the scientific, the technological, and the "humanistic-social." Since 1944, the Society has continued its study and implementation of this report, and as Dr. Gullette will show, there has been no significant change from the six major objectives established for the liberal arts portion of the engineering students' education. As in the three goals previously stated (which I personally prefer, probably because they are less definite), the engineering objectives are couched not in terms of subject matter but in terms of competences it is believed the humanities and social sciences can help their students to acquire:

1. An understanding of the evolution of the social organization within which we live and of the influence of science and engineering on its development.
2. The ability to recognize and make a critical analysis of a problem involving social and economic elements, to arrive at an intelligent opinion about it, and to read with discrimination and purpose toward these ends.
3. The ability to organize thoughts logically and to express them lucidly and convincingly in oral and written English.
4. An acquaintance with some of the great masterpieces of literature and an under-

standing of their setting in, and influence on, civilization.

5. The development of moral, ethical, and social concepts essential to a satisfying personal philosophy, to a career consistent with the public welfare, and to a sound professional attitude.
6. The attainment of an interest and pleasure in these pursuits and, thus, of an inspiration to continued study.

These are reasonable and practical goals to have for four undergraduate years. Many of us are convinced that they are not only capable of being achieved, but that they are a practical necessity if our education is to have the direction and the purpose it must have if the race is to survive. The problem ahead of us is really not that of knowing what we ought to do but of doing it. That is the subject that Dr. Gullette proposes to approach. Let me wish him and you the glories of success in this venture—long life and happiness.

THE PLACE OF THE HUMANITIES AND SOCIAL SCIENCES IN A TECHNICAL CURRICULUM

V. A. Rice[1]

School of Agriculture, North Carolina State College, Raleigh

The question as to what place the humanities and social sciences occupy in dairy husbandry curricula is, for the former, easily answered. The answer is none. Most curricula do include some social studies such as economics, sociology, and political science and most of them, too, probably include some history which in some minds is classified as social science; in others, perhaps among the humanities. But there is at least a paucity, if not a complete absence, of literature, philosophy, ethics, logic, art, and music.

In the early days of agricultural colleges, the curricula were, for the most part, composed of liberal arts subjects because *(1)* there was no body of technology and science in the field of agriculture, and *(2)* the faculties consisted of men who had graduated from liberal arts colleges, the only colleges then in existence.

Catalogues of the early agricultural colleges almost invariably contained statements such as the following: "It is intended to train young people by giving them not only a liberal but also a special education" and "It is believed that every young man needs a double education, one that is practical, to fit him for his profession, another that is cultural, to fit him to live."

Following the establishment of the experiment stations, the flood of scientific discoveries began to rise. Since college maintained its original time limit of four years of approximately 36 weeks, something had to give, and about the only thing that could give was the Liberal Arts. The flood of science has, as you know, now eroded practically all of the humanities, so that our students today get their literature from *True Confessions* and the like, their art from the comics, and their music from the juke box, with the hodge-podge of TV thrown in for good measure.

At their founding, most agricultural colleges had one Professor of Agriculture and, if a good one, he was well-versed in what was then known in Soils, Crops, Animal Husbandry (including Poultry), Agricultural Economics, Horticulture, and Farm Machinery, such as it was. No need to remind this audience how that has now so completely changed, with specialists in a dozen or more fields within many departments, and divisions within departments.

It seems eminently fitting that we pause for a short time during this 1958 meeting of the American Dairy Science Association and ask ourselves the question as to whether the purpose of our type of education as originally set down was a sound one, to consider how far we may have strayed from it and what, if anything, we can do about getting back onto the right track.

A prime question is "Can we, in four years of college work, give a man a start toward a liberal education and at the same time train him for a profession?" How happy and satisfied liberal arts colleges are with the product resulting from their four years of intensive liberal arts training we do not know, but we would be surprised if complete satisfaction is their lot. Likewise, specialized technological colleges, giving at best a very bare minimum in the humanities, probably feel that lack of time has not permitted the degree of specialized achievement they would have hoped for. How, then, can we who try, after a fashion, to do both jobs at once, hope to achieve any great degree of success? This is asked not to encourage discouragement but hopefully, to challenge more intensive diligence to a very difficult job. The eventual achievements of many of your own graduates, both as well-

[1] Visiting Professor.

rounded men and as highly skilled technologists in their fields, indicate that the job can be done, at least with certain individuals.

To start, we should perhaps ask the question as to what sort of person we would like our graduates to be—in short, what sort of chap is a liberally educated technician? What is the meaning of a liberal education? Many volumes, embodying a host of ideas, have been written on this topic. Two of the best ones which the present writer has come upon are "An Open Letter to College Teachers," by Payne and Spieth, and "The Meaning of a Liberal Education," by Everett Dean Martin.

We can start by listing a few things that education is not. First of all, education is not propaganda. Colleges exist, or should exist, for the purpose of helping students to learn how to think, not what to think. College work should not be passive acceptance but active doubt and criticism. Next, education is not animal training. True, we should strive to graduate people who can do things; give a talk, manage a herd, run a pasteurizer, etc., but dogs, too, can be trained to do tricks. We want capable doers, to be sure, but we should also want them to know the significance of what they do, to have a delicate discrimination for the best in all fields, to be free from vulgarity and bigotry, and with their judgments determined by something other than passion and prejudice. Animal training may give one the means of making a living, but it won't necessarily give life a meaning. Our graduates should be capable technicians in their respective fields, but they must also have some inkling of how to live a worthwhile life and, hopefully, also know how to take part in community activities.

Education is certainly not book learning—so many years of this or that. Books—yes, even colleges—can provide the means for acquiring an education, but they are not of themselves that which should be called an education. A good college certainly will implant good habits of discriminating reading in its students. Our schools have wiped out illiteracy and what does the general public read? Mostly trash. How, other than by reading, can a person become acquainted with the great ideas of religion, ethics, government, economics, and science which are responsible for the gains which man thus far has been able to register in his struggle up from savagery? The writer has been puzzled for forty years over so many students' reluctance to take any courses in the field of literature, beyond those prescribed. Apparently, many English departments could improve their techniques for instilling a love of good books in the hearts and minds of our students.

Space forbids foraging further in the extensive field of what education is not, except for a short quotation from Martin: "When all is said the ignorance and folly of men are things that institutions cannot cure. Each must discover the path of wisdom for himself. One does not 'get' an education anywhere. One becomes an educated person by virtue of patient study, quiet meditation, devoted to the discovery and service of truth."

Now, on the positive side, what is an educated person? Each would phrase his definition in his own way, but included most certainly would be the idea of freedom—a mind free from prejudice, from superstition, from bigotry, from dogma; a mind with the courage to accept and accommodate anything which can be proved true, regardless of what the new facts may do to the old, cherished ideas; and, in addition, a mind so trained and fortified that it is able to discriminate the best in all human endeavors.

We leave to others the courses and sequences required to begin the job of freeing, emboldening, and sensitizing the minds of our students, although insisting that some courses in literature or ideas, and at least one course in philosophy, must in some way be included in every student's course of study. Perhaps it will turn out that the problem is men rather than courses, although in our hearts I believe we know it to be a matter both of men and of materials. It must indeed be a sorry college or university which does not number among its faculty at least a half a dozen men with whom dairy husbandry students should have a course, regardless of what these teachers might happen to be teaching.

In a lecture on education to be found in a book called "Lectures and Lay Sermons," Thomas Huxley defines an educated person in the following manner: "That man, I think, has had a liberal education who has been so trained in youth that his body is the ready servant of his will, and does with ease and pleasure all the work, that as a mechanism, it is capable of; whose intellect is a clear, cold, logic engine, with all its parts of equal strength, and in smooth working order; ready, like a steam engine, to be turned to any kind of work and spin the gossamers as well as forge the anchors of the mind; whose mind is stored with knowledge of the great and fundamental truths of Nature and of the laws of her operations; one who, no stunted ascetic, is full of life and fire, but whose passions are trained to come to heel by a vigorous will, the servant of a tender conscience; who has learned to love all beauty, whether of Nature or of Art, to hate all vileness and to respect others as himself."

Huxley, of course, was a fighter—he bore the brunt of the attack against the idea of evolution propounded by his colleague and friend, Charles Darwin. In the above quotation, his polemic was with the humanists of his time who were fighting the inroads of science into formal, liberal education. We should like to interject one item left out by Huxley, by adding after his sentence, "whose mind is stored with a knowledge of the great and fundamental truths of Nature and the laws of her operations": as well as with the great literature of

ideas and the wisdom gleaned by man in his endeavors to read meaning and value into his understanding of the universe and his fellow man.

What each of us can do, must do, if we are to be true to the trust placed in our hands is to see to it that our own education, cramped as it probably was by our college days, grows in depth and breadth with each passing year, or better, day. We must teach our own special courses, scientific or technological for the most part, perhaps, but there should be ways of making any material liberal. In the final analysis, it is a matter of how we teach, not what we teach. Those of us who have been long immersed (or submerged) in the art of teaching have finally come to realize that we teach more by indirection than otherwise. Boys will remember what we are rather than what we teach. In the field of technology they must soon forget what we teach them today, for tomorrow new techniques will have replaced today's best methods. An English Dean at Oxford once said that "Education is that which remains when all that which has been learned has been forgotten." In short, education is an intangible something that a boy gets through close association with his teachers. They are learning every minute they are with us, perhaps only 70% of what we think we are teaching, but close to 100% of what we are. Actually, the teacher's job is a very easy one to state, namely: that of inspiring students to want to learn, arousing their intellectual curosity. If we do that, we are successful, regardless of what department heads, deans, or presidents may believe. Are we well-read, broad-gauged, tolerant, intellectually curious, unprejudiced, and fair? If not, there should be no place for us on a college faculty. Is it our aim to help students to learn how to stand on their own feet, to delve deeply and critically into any subject, and to have the courage of their own independent, disciplined, and discriminating convictions? Or do we think we should indoctrinate our students by spoon-feeding in homeopathic doses? Are we grieved over an athletic defeat and complacent about whether our college is creating an intellectual ferment in the minds of its students? If our labors do not open students' windows onto a wider, higher universe and broaden and deepen their outlook, their sympathies, their understanding of the world and their fellow men everywhere, we have not only failed in our present function but probably our students have missed their last chance to begin the life-long task of acquiring a liberal turn of mind. To us has been given the most awesome responsibility of any class of men—the opportunity of fertilizing the minds of boys, so that they may eventually grow into liberal men. Again, I say subject is unimportant; it's simply a question as to whether we ourselves are struggling up that hard road toward a free, courageous, disciplined, and discriminating mind.

If we are, we can stop occasionally, draw a deep, satisfying breath, feel the glow of an inner contentment that we have not been untrue to the greatest trust a man can have—the nurture of God's highest creation—the mind of a boy. And we can feel a comradeship with all great departed teachers such as the late Dean Hawkes of Columbia who wrote: "If we are successful in bringing up able men and women who are

> Competent in mind
> Vigorous in body
> Tolerant in attitude
> Courageous in spirit and
> Cooperative in temperament

we shall have reason to speak our *Nunc Dimittis* with composure."

HOW TO DEVELOP A LIBERAL STUDIES PROGRAM IN AGRICULTURAL EDUCATION

G. A. Gullette
Department of Social Studies, North Carolina State College, Raleigh

Our part in this discussion will be to consider some of the practical problems that must be solved if the goals and objectives that Dean Shirley has outlined are to be achieved without seriously weakening the technical and scientific aspects of agricultural education. Everyone would agree that if there were money enough and time enough, we would like to see all young people, and especially the ablest among them, push as far as their capacities would permit them in every field of human knowledge, including the humanities and social sciences. This might take more than one lifetime, but, if we didn't have to worry about that, we might all agree that it would be a good thing.

The simple truth is that we don't have that amount of time; economic forces compel early and thorough specialization; and the practical question in the real world is whether anything significant can be done to broaden the outlook of technical students in a four-year undergraduate program, while leaving adequate room for the scientific, agricultural, and business training that is so necessary.

The answer to this question is an emphatic "Yes!" It not only can be done, but it is being done in the leading engineering schools in the country, which face a problem just as acute as that of the colleges of agriculture.

It is known from first-hand observation that a reasonably successful job can be done if only four courses—that is, four full-year courses—are allotted to the program, and most agricultural curricula now allot something close to this amount, but students are not getting a full return for the time invested because certain conditions are not being met.

First, no liberal studies program is going to do any good unless it kindles the interest and fires the imagination of the students who are taking it. This is not likely to happen if students are required to take, or even allowed to elect, courses vaguely thought to be broadening because they are labeled History or Philosophy or Literature. The necessary enthusiasm can be kindled only if the courses are planned to meet the needs of, and the interests of, the students who are taking them. They must also, of course, be taught by individuals who are not only competent in their own fields but who have some genuine understanding of agricultural students and their problems. You are certainly not going to get much help from a liberal arts instructor who believes that anyone who decides to study agriculture automatically resigns from the human race. The first condition for a successful liberal studies program, then, is that it should be planned for those who will take it and be taught by men who are sympathetic.

The second condition is that the courses should follow a planned sequence extending throughout the four undergraduate years. This is a simple-seeming device that was invented by the engineers, and it is the most significant development for general education in our time—particularly significant for technical schools. This vertical sequence idea accomplishes many things. Because the student is in contact with the humanities and social sciences from the day he enters until the day he leaves college, he gets over the idea that these courses are something to be gotten out of the way as quickly and painlessly as possible, so that he can go on to more important matters. He begins to see them as related parts of his whole education, and it becomes, incidentally, easier for the instructor to show him relationships with his other studies.

Furthermore, the four-year sequence can take advantage of the growing maturity of the student as he becomes an upperclassman. Our experience with engineers clearly shows that when they become juniors and seniors they begin to see the importance of a broad education in a way that they never did as freshmen and sophomores.

But most important of all, the sequence permits the systematic development of a set of ideas over a period of years. Each course can build upon its predecessor, giving a coherence and depth that no collection of introductory courses from various fields could hope to give. One of the charges made against general education courses is that they are too superficial, but the sequence eliminates this danger. The second condition, then, for a program of liberal studies for technical students is that it should be arranged as a vertical sequence of courses running throughout the four undergraduate years.

The third condition follows as a necessary consequence from the first two. Since the courses must be planned to meet the needs and interests of a certain kind of student who will never be a major in the fields of the humanities or social sciences, and since the courses must be related to each other in an ascending order throughout the four years, it follows that no mere juggling of traditional courses is going to meet the needs of agricultural students. The courses must be reorganized, recast, and, in many instances, completely rebuilt on a new plan. And there is the problem.

For those of you who are in education, there is no need to explain further. For those of you not in the academic world, getting a college professor to change one of his courses is a tremendous undertaking, and if you should ask that he work with a group of other college professors to develop a sequence of courses that make sense together, you must be prepared for howls of pain and anguish, to say nothing of threat to life and limb. Someone has said that trying to change a college curriculum is like trying to move a graveyard and the analogy, while not perfect, is at least suggestive of some of the difficulties.

However, difficulties are not insurmountable. The task of building a program of liberal studies that will make sense for students of agriculture, and accomplish some of the aims that Dean Shirley has sketched out, is not easy, but it is certainly not impossible. Engineering schools throughout the country have proved this beyond any possibility of quibble. What is required is a clear understanding of what it is you are after, and a firm determination to get it, within reasonable human limits.

Three basic prerequisites to a successful program of liberal studies for technical students have been mentioned. Two other practical matters are worth mentioning. First, we know from our study of engineering schools that the difficulties of developing and sustaining a vigorous program are so great that the whole thing can bog down in mediocrity unless it has the enthusiastic support of both the technical faculty and the liberal arts faculty. This support, in turn, can not be achieved if the planning and supervision of the program are unilateral—that is, if the job is turned over either to the liberal arts faculty or to the technical faculty. The two must work closely together on the program from the beginning, so that both groups feel they understand each other and have a joint stake in the success of the undertaking.

Secondly, a program of liberal studies is bound to disappoint its sponsors if they fall

into the trap of confusing the humanities and social sciences with business training. Courses in accounting, business law, labor-management relations, and the like are entirely legitimate as a part of one's professional training, but they will not serve the purposes for which a liberal studies program is designed. When we suggest that it is entirely possible to give the hard minimal core of a liberal education in four consecutive courses, we are thinking of four courses that will devote themselves exclusively to that central goal. We are not thinking of just any courses that happen to be taught by the liberal arts faculty.

We have tried to indicate here briefly, but realistically, our basic conviction that vocational, technical, and semiprofessional students in American universities do not have to sacrifice the central values of liberal and social studies merely because they are forced to specialize early. We are quite aware that many people disagree with us, but we think the facts are on our side and are prepared to cite them. What is required, basically, is a change in our traditional view that liberal education is something that precedes specialized and professional education. In our view, it should not precede but should accompany such education, and when it does so, both the special and the liberal aspects of education receive new strength.

THE CURRICULUM IN DAIRY MANUFACTURING

Broadening the Curriculum, Technology Courses Versus Science Courses, Process Courses Versus Product Courses

F. J. Doan
Department of Dairy Science, Pennsylvania State University, University Park

We have been asked to present our views briefly on three topics pertaining to instruction in dairy manufacturing or dairy technology:

1. Broadening curriculums
2. Technology courses versus science courses
3. Process courses versus product courses

It can be assumed, to begin with, that no program in dairy technology possibly can be arranged which will satisfy everyone interested in dairy manufacturing education, least of all the students who are taking or contemplate taking such a program.

In any discussion of curriculums, the matter must be considered in the light of certain background facts and information which are pertinent to the subject.

In the different regions the dairy industry itself varies in complexion, and it is natural to suppose that dairy departments will tend to emphasize areas of instruction which seem to fit the opportunities offered by industry in their regions.

No hard and fast curriculum of studies can meet perfectly the needs of all dairy technology graduates, for the simple reason that the positions available to them are too diversified, running all the way from scientific research of basic character to selling supplies, or from managing a complex dairy operation to inspecting farms and plants. We have alumni owning, managing, and working in dairy plants; as laboratory workers and inspectors with health agencies; as fieldmen and procurement people for dairy companies; as technical and economic advisers to cooperatives; as laboratory directors and personnel; on research, teaching, and extension staffs with state and federal institutions and agencies; as market administrators; as designers, service men, salesmen, and troubleshooters with equipment manufacturers and supply houses, and in many other and less obvious types of employment. No set curriculum possibly can be ideal for all these areas at one and the same time. If a student's adviser knew in advance what area of employment the student would enter on graduation, a better curriculum could be devised, or better preparation could be had, by the judicious selection of elective and optional courses for his program. But this is seldom known.

In recent years, we have heard much bewailing of the fact that enrollments in dairy manufacturing have been decreasing. It can be pointed out also that the number of dairy plants and the number of industry units also have been decreasing, even though the unit size has been getting larger. There is good reason to believe that because of this tendency and the accompanying trend toward large-scale continuous flow processing, process automation, automatic controls, and so forth, the number of positions in dairy plants open to dairy manufacturing graduates is becoming fewer. Therefore, some decrease in enrollment may be justifiable. It also may be true that an increasing proportion, or even a greater number, of the available openings to graduates are outside of plant and production work. If this is true, some modification of curriculums may be desirable to compensate for this trend.

Students occasionally have desires and ambitions which are not matched by their aptitudes, capabilities, or aggressiveness. For example, we frequently have students tell us in their

junior or senior years that they wish to take graduate work, when their scholastic records and curriculum content would militate against their acceptance in a graduate school. In such cases, it is necessary for the adviser to try to direct the student's interest toward other, less demanding, objectives.

A more common occurrence, however, is the student who has the ability and who could carry a heavy scholastic load successfully, but who is content to follow the lines of least resistance, avoiding "tough" courses and feeling satisfied merely to get by. This is a reflection of the attitude prevailing in our school systems, especially among teen-agers, where the student, or scholar, is commonly regarded as a "square." As you know, a great deal of concern is being manifested throughout the nation relative to this attitude at the present time, and it appears that there is hope for a swing away from some of the more objectionable aspects of the philosophy of progressive education, which seem to be at least partly responsible.

College curriculums in technical education have frequently been criticised as trade school programs, and institutions of higher learning are being admonished to elevate their standards. It is our feeling that colleges should not squander time in the attempt to give students mere experience or practice in processing, operating equipment, or manufacturing products. The function of the college is to teach fundamental sciences and arts; to familarize the student with the application of the fundamentals to the various activities and operations of industry, in all its phases; and to stimulate the student's ability to reason inductively and deductively from the learning he acquires. Practice and experience should come through actual work. An employer should not expect a graduate to be a highly skilled operator, any more than he would think of making a graduate of a business course his head accountant. Industry, however, should expect a graduate to become quickly competent in almost any phase of its operation because he is familiar with underlying principles.

The suggestions that have come from industry regarding the composition of the curriculum in dairy technology are about as varied as one could envision and, in composite include, besides a good broad general education and competent training in basic sciences and technology, considerable work in such areas as mechanical engineering, business, public relations, personnel and labor problems, cost accounting, industrial efficiency, selling and merchandising, and others. Obviously, it is impossible to work all of this into a four-year program.

Broadening Curriculums in Dairy Manufacturing

College programs in many curriculums, including dairy manufacturing, are often criticised as being too specialized or narrow, and lacking breadth of outlook in areas of general education.

Ideally, it would be pleasant to think that our colleges could turn out a man completely trained in his specialty or profession and at the same time at least familiar with all other facets of knowledge. In the time available, this is manifestly impossible. So we compromise, and maintain that the professional or technical student should be required to take a certain number of courses in subject matter areas outside his major interest, to broaden his education and make him familiar with and sympathetic to other areas of learning. What these courses should consist of is a very puzzling question.

Common sense would seem to dictate that these oftimes-termed cultural courses should be in subject matter that would equip the student to understand the economic and social facts of life more adequately, to exercise his privileges and duties as a citizen more intelligently, and to live more abundantly. These objectives, of course, again cover an impossible area, particularly the last one which, conceivably, might include almost anything from anthropology to fly-tying.

It is our feeling that a curriculum in dairy manufacturing, in common with most agricultural curriculums of a technical nature, should consist of a hard core of required courses in mathematics, chemistry, physics, biology, English or communication, bacteriology, economics, and possibly psychology. This basic group of studies should make up about 40% of the total baccalaureate program.

Required courses in dairy manufacturing or technology, including applied bacteriology, chemistry, and engineering possibly should add another 25%, which would increase the total to roughly 65% of the program.

About 12 to 15% of the program might include supplementary courses which would contribute to a student's competency in his chosen major or broaden his knowledge and capabilities in allied fields. These courses might be selected from a list, or scheduled after consultation with an adviser. Essentially, they would be electives, and at least some of them would classify as broadening courses. They would bring the program total to from 77 to 80%.

In state institutions, two years of military instruction and physical education commonly are required. The amount varies some from place to place, but averages about 7% of the program.

Quite a number of colleges in recent years have become sensitive to criticism that technical and professional graduates are woefully lacking in general education and cultural appreciation. As a result, some of them have made it obligatory for students in these areas to schedule from 6 to 8% of their program in studies variously referred to as the humanities, citizenship electives, cultural studies, etc. The subject matter permitted here generally is quite broad and any student most certainly would find worthwhile and beneficial courses in the

group. Nevertheless, most courses available in these areas are as specialized as in the professional fields. What are really needed are well-taught "appreciation" courses or survey courses of considerable latitude, which relate the subject matter to other areas of knowledge and human thought or practice.

At this point, we have circumscribed 90 to 95% of the student's program. The remaining 5 to 10% might well consist of free electives to be taken with the approval of the adviser. The adviser should not attempt to dictate courses in this group, but should merely satisfy himself that the student is sincere in his choices and will make good use of his time.

It is our belief that a curriculum in dairy technology can be set up, on the basis outlined, to accommodate the needs of any student seriously interested in employment in the dairy industry, in any of its phases and in most of its allied endeavors. Moreover, the program will furnish a fairly good scientific background, a fairly adequate general education, and a reasonable proportion of broadening courses in other areas.

As an example of how such a curriculum might fit the needs of two students with widely differing objectives, let us first consider a man who desires an exceptionally good science background because he expects to take graduate work and wants eventually to become a research worker in dairy or food products. By proper selection and election of courses, he may reinforce his program by using the supplementary and free election groups of studies for additional chemistry, physics, mathematics, bacteriology, etc., to the extent of about 20% of his total program.

In the same manner, a student whose family operates a dairy company and who reasonably expects to take over the business at a later date, can similarly fortify his program with a like amount of additional work in such areas as accounting, business law, insurance, labor problems, economics, management, and public relations.

These are extreme cases. The average student who is not sure of his future may hedge, and select some subjects on each side of the fence, hoping to be reasonably well-grounded in science and technology as well as in economics, business, and management.

TECHNOLOGY COURSES VERSUS SCIENCE COURSES

Students majoring in dairy manufacturing usually are required to pursue a number of courses taught in dairy departments dealing with the manufacture, processing, handling, and quality control of various dairy products. The question involved in this subheading, as we interpret it, is: Would a student be better prepared for employment and work if he spent more of his time in background science courses: chemistry, physics, mathematics, bacteriology, nutrition, etc., and less time in applied technical work? This question, we think, can not be answered positively, because of the variables concerned. Curriculums differ greatly in different institutions, as do available course offerings. The desirable qualifications for the many opportunities open to graduates are by no means uniform. Moreover, what might be highly suitable for one student might be totally unrealistic for another. It must be remembered, too, that in most cases neither the student nor his adviser knows what type of employment the student may accept upon graduation.

Certainly, as we have indicated previously, all students should be required to take basic courses in science and general education, representing something like 40% of the program, to which may be added applied or specialized scientific courses like food chemistry, food bacteriology, and food engineering. Whether further scientific work should be added at the expense of dairy technology courses (product or process) is highly debatable and, personally, we would have to be convinced by rather strong arguments.

What this question really infers is that curriculums in dairy manufacturing are outmoded and should be superseded by curriculums in general food technology, food chemistry, bacteriology, and engineering, or merely basic biology, biochemistry, and bacteriology, with industrial emphasis. Frankly, we see no great advantage in this move at present, provided dairy manufacturing curriculums contain a reasonably satisfactory content of science courses.

Only a small proportion of graduates actually work in areas where a high level of science education is vital, but a large proportion work in areas where an intimate knowledge of milk, its products and their handling, processing, and peculiarities, is essential. Furthermore, a significant number of graduates are employed in management, business, and selling areas where science backgrounds are of less importance than economics, commerce, business, and marketing education, but even here a good workable knowledge of milk and dairy products is desirable, to say the least.

We do not think we are ready to substitute general science courses for instruction in dairy subject matter. However, we do feel that there may be some consolidation, elimination of duplication, and general upgrading possible in dairy courses as now taught. This is a matter for intensive and conscientious study on the part of the faculty of the departments concerned.

PRODUCT COURSES VERSUS PROCESS COURSES

Should the subject matter in dairy manufacturing or technology be taught as product courses, such as "market milk," "ice cream," "cheese," etc., or should it be presented to the student in the form of process courses like, "heat treating dairy products," "washing and sanitizing dairy equipment," or "fermentation and coagulation in dairy manufacture"? There are, of course, any number of ways in which the procedures, processes, and techniques of the indus-

try might be combined or arranged, none of which would be wholly satisfactory, any more than the division of subject matter into product courses is above criticism.

There are many in the teaching profession who believe that a more logical treatment of a process or technique, together with the equipment and machines for applying it and the effects of the process on products, can be obtained by considering it, in all its aspects, in one comprehensive dose. They maintain, for instance, that the subject of pasteurization must be covered in almost every product course and that this involves considerable duplication and wasted time; whereas, it could be concentrated in a process course, to cover all products and all aspects of its application. There is considerable merit in this argument, especially for certain processes.

On the other hand, a logical sequence of process courses covering all the important subject matter in dairy manufacturing is far from easy to organize. There is always the important question of which should come first, the chicken or the egg? Can heat treatments be discussed exhaustively with reference to all products before presenting fermentation and coagulation? Can it be done satisfactorily in reverse fashion? It seems to us that, either way, some inclusion of subject matter properly belonging in the other category would be necessary for a full understanding of the matter being presented. This would mean that avoidance of duplication would be as much of a problem with a curriculum of process courses as it is with product courses. It hardly seems likely that the subject of pasteurization could be discussed without a consideration of milk-borne diseases, sources of infection, public health supervision, the hazards of toxins, etc. and, if these are included, the process course soon ceases to be one.

Another difficulty with process courses is what to do with treatments, phenomena, and specialized operations which do not fit into any general process or over-all technique. Where, for instance, shall we locate such subjects as the washing and working of butter, the whipping of cream, or the making of process cheese and cheese spreads? Would it be necessary to set up a hodge-podge, catch-all course to take care of the odds and ends which are left over after the principal process courses have been delineated? If so, where should this course fall in the sequence?

A final disadvantage of process courses is that most texts and literature deal with products rather than processes.

The problems raised by these questions, of course, are not completely insoluble, but they are difficult and, when solved to the best of our ability, the curriculum made up of process courses seems unlikely to present the subject matter to the student any more understandably than would a curriculum of product courses. Naturally, a great deal depends on the ability of the staff, the facilities available, and other variable circumstances.

Product courses have the advantage of concentrating in one course the subject matter pertaining to a given product, not scattering it through a series of courses. In future years a graduate will be working, for the most part, with products, not with broad concepts and all-inclusive processes. There definitely will be some advantage for him to have all his information in one place for reference.

It is certainly true that product courses are apt to be repetitious in certain areas. This, however, is not so much a criticism of product courses as it is of the staff teaching the courses. Unnecessary duplication can be eliminated by a conscientious integration of material. It might be pointed out that some duplication of subject matter is not undesirable. It helps to emphasize important things, and different instructors frequently approach subjects from different viewpoints, thereby giving the student a better appreciation of all the aspects of a subject.

In conclusion, it is our judgment that it makes little difference whether dairy technology is taught by process courses or product courses, so long as the field is properly covered. A matter of much greater moment is the ability, knowledge, enthusiasm, and conscientiousness of the instructor. No course is any better than the instructor who teaches it. In the past, some of us have been blinding and deluding ourselves with paper outlines of courses and arrangement of curriculums, excellently described and displayed in our college catalogs, but which we then turn over to incompetent instructors and wonder why the results do not measure up to expectations. This criticism is by no means limited to dairy technology instruction. I think it is perhaps even more true in some other areas of college instruction. More attention should be given to the appointment of staff members who are expected to teach and to their ability to teach rather than to their potentialities for research or publications. Not many graduate students are capable instructors. Not all good research people are good inspectors. Some teachers are poor instructors. A curriculum in dairy technology stands or falls on the quality of instruction received by the student all through the college program.

TRAINING STUDENTS FOR LEADERSHIP IN THE DAIRY INDUSTRY

J. Hoffman Erb

Vice-President, Mid-West District, The Borden Company, Columbus, Ohio

During the last few years, there has been much discussion and concern about the preparation of tomorrow's leaders in the dairy industry. Challenging problems press upon all segment of our great industry, and we depend increasingly upon professionally educated people for aid in their solution. Many feel our educational capabilities have not kept pace with our needs. The traditional dairy manufacturing curriculum, although contributing much to the development of our industry in the past, now appears to be in need of drastic overhauling.

The criticism has frequently been heard that too many of the graduates from the four-year dairy manufacturing programs do not seem to have acquired the kind of basic preparation that gives them the ability to grow and develop into responsible positions of executive leadership. It is a healthy sign that educators, as well as industry leaders, are aware of the general problem. Both groups share the responsibility for providing a proper organization and content of the educational structure. The aims of dairy education must be clear, and plans to attain these aims must be carried out by the faculties of our universities.

The commercial dairy industry is foremost among the groups looking for better educated men. We continue to have constant need for scientists and engineers capable of maintaining and advancing our technology but, in a more pressing way, we are confronted with a growing need for young men to develop into positions of leadership capable of handling the complex affairs of the modern dairy enterprise. We shall enlarge on the desirable qualities necessary in executive leadership for our industry. Analysis of this one facet of need may give a clue to the educational framework which is needed.

The vital requirement in a successful business enterprise is the executive, or administrator. Primarily, he directs the activities of other people and assumes responsibility for achieving certain objectives through the efforts of these people. The success of the administrator rests on three basic skills—technical, human, and conceptual. In the preparation of young men with special technical knowledge of our industry, the four-year university programs have, in general, been doing a good job. This is the most concrete and relatively easiest skill to master. We must see to it that our students continue to acquire a thorough understanding of fundamental knowledge and a familiarity with the basic techniques in dairy chemistry, bacteriology, and related fields. But technical training is not enough.

Executive skill in human relations is the ability to build cooperative effort with the team the executive leads. This skill involves an understanding of people's attitudes, assumptions, beliefs, and sensitivities to the behavior of others. Today, industry responsibilities are most often carried out in group relationships. To live and work with one's associates, to have their respect, and abide by the group decision are qualities essential to success. Human skill is important in every level of supervision.

Finally, the good executive must possess a perspective of the enterprise as a whole. This is sometimes called conceptual skill. His acts and decisions must be such that they advance the over-all welfare of the organization. The action is broad-scale. In the final analysis, overall success is most dependent on the executive conceptual skill.

It would be well for those in the dairy teaching profession to realize that dairy industry leaders can make up for a lack in technical skill if they are experts in human and conceptual skills. While a man's technical knowledge may be his best tool during his first five to eight years, this skill tends to have less value with the passing of time, and his skill in human relations and his conceptual skill rise sharply in value with the passing of years.

It seems that this brief review of the dairy industry leadership requirements accentuates the importance of minds trained to be creative, imaginative, and completely at home in new situations. In other words, to meet the needs of the modern dairy industry, technical training is not enough. The specialist can not function effectively in the role of management if all he brings to it is his specialty. To keep pace in today's world of business, wide-ranging minds are needed.

Our question then is, "Is there any kind of education which will equip our potential leaders to cope with such responsibilities?" For years, claims have been made that a certain kind of education has such results. It is education that seeks for meanings and emphasizes the power to discriminate. It is concerned about relationships and human values. This is usually called liberal education, or the discipline of the humanities.

Among other things, this category of education is heavy on social exploration, by which the student attains perspective and new insight of the world in which he lives. This area includes subjects such as fine arts, literature,

philosophy, history, economics, psychology, and political science. These subjects are good foci for the study of human relationships. When the instruction is good, they should enlarge the range of one's knowledge and experience.

Teaching should result in open-mindedness, breadth of outlook and, above all, in the balanced growth of the individual. Because of the limited time possible in a professional four-year program, one must guard against a superficial scattering of attention over a number of liberal subjects. The answer is for the student to select from only several of these subjects, and pursue one or two to considerable depth. It seems clear that in the future the scientific technological studies can not absorb the student's time as completely as has been common heretofore in many of our dairy manufacturing programs. We might well allow one-third or more of the credit hours to accomplish the broader aspects of the student's professional problems.

The need for a liberal education in the professional and technical field is increasingly recognized. The engineers have been among the first to do something about this. The nation's leading engineering schools are now introducing more of the humanities and social sciences into their educational programs. Massachusetts Institute of Technology and California Institute of Technology have been insisting on a good grounding in the socio-humanistic subjects before specializing in the engineering studies.

The basic aim of education in a specialized field, such as dairy manufacturing, should be to equip students to grow throughout their lives, both in the field of their specialty and in personal stature and usefulness as citizens. Students with training limited to the minutiae of dairy practices have a background of mere artisans and will not become professional men of full stature.

Business is coming to recognize that narrowly specialized training is precisely the wrong kind of training for men who will be expected to play a creative role in the age before us. The task is to educate people who can adapt to change. It is encouraging that some of our dairy industry educational programs are on the path of a broader and more liberalizing education. It is to be hoped that more of our universities which give this professional training will provide an opportunity for students to acquire an orientation, both in the field of scientific technological education and in the area of human values. The ends of the two divisions are complementary. Neither can provide training which is the function of the other.

Our professional dairy education could be likened to a rope composed of four strands running its length—the strands consisting of the basic scientific, the technical, the humanistic, and the social sciences. Implementing all of these subjects into a strong whole is the challenge before our educators.

OUR ASSOCIATION

REPORT OF REPRESENTATIVE TO THE UNITED STATES LIVESTOCK SANITARY ASSOCIATION

W. D. KNOX
Editor, *Hoard's Dairyman*, Fort Atkinson, Wisconsin

Since this is the first report of a representative from the American Dairy Science Association to the U.S. Livestock Sanitary Association (USLSA), a brief review of its history is appropriate.

On September 27, 1897, the operators of the Fort Worth Stock Yards invited state veterinarians of the northern states to come to Texas to observe a new method reputed to free cattle from disease-bearing ticks. Out of this meeting evolved an association devoted to the control of many diseases of domestic livestock, some transmissible to man.

From 1897 to 1909, livestock industry leaders and animal health officials met as the Interstate Livestock Boards and State Veterinarians. In 1909, the name was changed to the U.S. Livestock Sanitary Association. Since then the membership of the association has gradually changed from its original composition, chiefly of stockraisers, to its current membership, which largely consists of regulatory officials, veterinarians, scientists, and research workers engaged in animal health activities.

Each year, USLSA holds its annual meeting in November. The 61st annual meeting was held last fall, November 13-15, in St. Louis, Missouri.

Prior to its formal session, the association's many committees meet for two and three days and nights preparing reports on such subjects as tuberculosis, brucellosis, anaplasmosis, leptospirosis, and other livestock health problems. During the convention, numerous technical papers are presented by leading veterinary research workers. Considerable discussion follows each paper. In the late hours of the annual meeting, completed committee reports are presented, discussed, and then referred to the executive board, which is composed of the state veterinarians from each of the states and territories. Actually, it is now the executive board, and not the direct membership, which controls the policies of USLSA.

Down through the years, USLSA has evolved into an extremely potent agency. Because national efforts to control and eradicate infectious diseases are dependent upon cooperative action by the federal and state governments, the executive board of USLSA becomes the only coordinated voice of the states in livestock health matters.

This, of course, has placed USLSA in an extremely strong bargaining position, insofar as its relationship with the federal government is concerned. A state veterinarian is normally on the staff of the state commissioner of agriculture, who has close and intimate working relationships with state government, including his state's representatives in Congress. Since Congress controls the appropriations for the U.S. Department of Agriculture, it is easy to visualize how USLSA has become so influential, when it has maintained discipline within its ranks and has agreed on a policy position.

Indicating the scope of interest and activities of USLSA are the following standing committees:

Anaplasmosis
Biologics and Pharmaceuticals
Stockyards, Markets, and Transportation
Brucellosis
Laws and Regulations
Leptospirosis
Transmissible Diseases of Swine
Infectious Diseases of Cattle
Eradication of Hog Cholera
Legislation
Tuberculosis
Vesicular Diseases
Parasitic Diseases
Transmissible Diseases of Poultry
Public Health
Public Relations
Rabies
Regulatory Education
Resolutions

Naturally, not all of the activities of USLSA are of direct interest to the American Dairy Science Association. This report is limited solely to summarizing those activities which are of direct interest to the dairy industry.

Mastitis. Although the association does not have a committee on mastitis, it devoted a considerable portion of its 1957 convention to the subject. A panel discussion, moderated by Dr. M. G. Fincher of Cornell University, featured four participants. Their discussion follows:

C. D. Van Houweling of USDA cited the economic importance of mastitis. Milk losses from mastitis in 1956 were estimated at about five billion pounds, or a conservatively estimated monetary loss of $200 million. Nothing is known of losses resulting from lower income because of poorer quality or losses resulting from replacement of chronically affected animals. About three of every 1,000 cows die of mastitis, adding another loss of $8,500,000. Therefore, losses for 1956 totaled more than $245 million. Another loss results from the cost of the treatment of mastitis.

J. M. Murphy of Cornell University discussed the results of his research for the past 9 yr. in

New York state. He limited his observations to the four main causes of mastitis:

1. *Streptococcus agalactiae*
2. Other streptococci
3. Staphylococci
4. Bacilli

He stated that at least 99% of all cases of mastitis fall into these groups.

According to prolonged observations in New York, 50% of the cows are not infected with any pathogenic bacteria, 23% are infected with *S. agalactiae,* 13% are infected with other streptococci, 13% are infected with pathogenic staphylococci, and 1% are infected with bacilli.

Most infections are in the nonclinical stage and the cows might be classed as normal on the basis of ordinary barn tests. About 24% of the animals have mild clinical infections and about 1% have severe clinical infection at any given time. It is important to note that all four forms of mastitis can exist in each of the three stages.

S. agalactiae rarely causes severe mastitis and may be controlled with good sanitation and the proper use of milking machines. However, these measures alone would not eliminate mastitis, because we do not know how to stop mastitis before it starts. Little is known of the causes of any form of infection of the udder.

Dr. Murphy's work up to 1946 indicated that the host part of the host-parasite relation had a resistance that was extremely important. This had also been demonstrated in England, where it was observed that younger cows, not previously infected, were resistant to artificial exposure; whereas, older, previously infected cows quickly succumbed.

Of the mild injuries to the teat canals of resistant, first-calf cows only the removal of the soft keratin lining of the teats, in an effort to break the resistance, broke the resistance in every teat on which removal was performed. Abuses which did not destroy resistance included: (1) The repeated insertion part way into the canal of a small, sterile cotton swab, and (2) the repeated application of a milking machine for 10 min. at 17 in. of vacuum after all of the milk had been removed from the glands by normal mechanical milking.

W. D. Pounden, of Ohio Agricultural Experiment Station at Wooster, discussed the possible role of nutrition in mastitis. He presented data which provided no clear evidence of the definite role of nutrition in mastitis. He stated, moreover, that we lack an understanding of the inconsistencies in manifestations of diseases of the udder. For example, outbreaks may occur in one herd during one year but not in other years; or in one quarter and not others. In his opinion, a possible connection with the feed might be recognized in extreme problems.

Since periods of reduced resistance may occur, during which attacks of mastitis are more easily incited than at other times, a search for possible measurement of the resistance of animals was attempted. Logically, the examination of milk was in order, since bacteria must be active in milk to produce the disease. Milk samples were, therefore, checked to challenge the capacity of each sample to resist activity of various mastitis-causing bacteria. Capacity to resist acid production, according to Dr. Pounden, has provided the base for measuring the response to *S. agalactiae,* and the ability to resist change of oxidation-reduction potentials has been used with hemolytic staphylococci and coliform bacteria.

These methods may not prove to be actual indications of the resistance of animals to the disease; nevertheless, their application has frequently indicated trends that appear to parallel clinically recognizable variations. Results of tests have not pointed to the effect of feed on the relation between milk and the mastitis infections; however, milk samples had a lower average of resistance to hemolytic staphylococci in the summer than in the winter in 1954 and 1955 and, partly, in 1957. This was not true in 1956.

O. W. Schalm of the California Agricultural Experiment Station at Davis, presented a well-illustrated contribution on the relation of the milking machine to mastitis. He maintained that unless the milking machine and its use are understood, progress in the control of mastitis will be slow. Unless the cow yields milk into the teat, the milking machine can not operate. With freely flowing milk partly filling the inner chamber of the teat cup, the vacuum on the teat is below the maximum permitted by the vacuum controller, and the force of collapse of the liner against the teat is proportionately reduced. However, when no milk is in the teat cup the vacuum on the teat reaches its maximum and extends into the cavity of the teat. Schalm recommended:

1. Narrow bore, stretched liners on all conventional milking machines operated at 15 in. of vacuum.

2. Discard liners that have lost resilience.

3. Provide an air-admission hole in the claw to prevent surging of milk in the claw and teat cups from the milk hose.

4. Design teat cups to provide visual milking.

5. Interrupt flow of constant vacuum to teats and simultaneously stop action of pulsator on the liner before removing milking machine.

Brucellosis. For the past 10 yr., the writer has been a member of the Committee on Brucellosis of USLSA. This committee has been, by far, the most active committee in the association, has consistently held the largest hearings, and has exercised greater influence on the course of animal health than has any other committee. In 1957, among the papers presented at the convention and among the data considered by the committee, were the following:

Federal-state cooperative brucellosis eradication program, by C. K. Mingle, USDA.

Preliminary results on the whole-blood test for brucellosis, by Paul Boulanger, Quebec, Canada.

Résumé of brucellosis meeting held in Peru, by C. A. Manthei, USDA.

A summary of the whey-plate test studies in Minnesota, by M. H. Roepke, University of Minnesota, and now of USDA.

In addition, the committee heard a special report on the whey-plate test from Dr. Cameron of the California Veterinary Medical School. As a result of the hearings, the following recommendations (in summary) were offered:

1. Continued study of the whey-plate test is necessary before its value can be accurately determined.

2. Further research is essential to determine standards in conducting and interpreting the milk-ring test.

3. Measures should be taken to restrict the use of the shield and V vaccination tattoo to official work performed under supervision of a veterinarian.

4. The National Swine Council was invited to canvass its membership regarding brucellosis control, with emphasis on suggested regulations on interstate and intrastate movement of swine which may be infected.

5. Changes in the uniform methods should be considered with attention to adopting changes that more accurately reflect the intentions of the code.

Tuberculosis. Impartial evaluation of the national bovine tuberculosis eradication project indicates that little progress is being made in total eradication, and that the program hardly can be said to be holding its own. Current testing of the more suspected herds, and improved tracing of diseased animals to point of origin, can not explain the fact that the percentage of animals reacting to the tuberculin test during 1957 was higher than for any similar period since 1950.

Despite consistent setbacks, the federal-state cooperative goal continues to be total eradication. To this end, consideration has been accorded five problems:

1. Unsatisfactory procedures for identifying cattle, and for tracing those found diseased to point of origin, have prompted the committee's recommendation that the association establish special projects in each of the eight U.S. areas established by USDA to solve the problem. Specific conditions in different areas must be considered in relation to the over-all national indentification picture.

2. Although re-accreditation of areas, based on standards of efficiency in tracing cattle found tuberculous at slaughtering establishments, is a necessary adjunct to the eradication program, implementation of such rules should be deferred until trace methods are fully worked out. The Committee on Tuberculosis was opposed to less testing in areas where incidence is relatively high.

3. Although current laws restricting the sale of dairy products to herds free from tuberculosis are valuable, the interest of uniformity demands code amendments mutually satisfactory to officials of the U.S. Public Health Service and USDA. Proposals to this effect were requested to be presented to the Livestock Sanitary Association in 1958.

4. The no-gross-lesion reactor is considered a problem in many areas. Further studies, with a view to carrying on additional investigations and research, in an effort to find means for reducing the severity of this problem, should be pursued (USDA is doing so).

5. Veterinarians accredited for tuberculosis eradication work are frequently inexperienced and inadequately trained, reflecting on policies and procedures of state and federal livestock sanitary officials. Cooperating officials should assure themselves that veterinarians recommended for accreditation are sufficiently trained and indoctrinated to perform their duties properly.

Leptospirosis. In the United States, leptospirosis was first confirmed in domestic animals in 1944, although histopathological and serological evidence indicates that it was present before that date. The causative organisms were isolated in 1946, stimulating many research projects.

After an incubation period of from seven to ten days, the more important manifestations of the disease in dairy cattle are: acute febrile illness, loss of weight, declining milk production, and possibly hemoglobinuria to a chronic illness. During third trimester of pregnancy, abortion is frequently observed.

Accurate history and clinical signs form the basis for diagnosis in cattle. Abortion strongly suggests the disease, particularly if other causes, such as brucellosis, can be ruled out. Use of serological tests is indicated, since leptospirosis is a herd problem. Almost all existing tests are valuable, but Stoenner's rapid-plate agglutination appears to be the most practical. The use of this test, or a superior one if developed later, by diagnostic laboratories must be considered the first step in any control program.

The full impact of leptospirosis can not be ascertained until more adequate reporting systems are developed, despite the fact the disease has been reported in every part of the United States and Canada. Dramatic results are not now available, regardless of therapy. Antibiotic therapy, and the usual supportive measures, are of some help, although too expensive to receive serious consideration at present. Reliance must be placed on vaccination rather than treatment of carrier animals. Commercial vaccines produced immunity for 1.5 yr. in cattle; sometimes for 6 mo. in swine.

Since control must proceed on a herd basis, isolation and vaccination provide the backbone of any control program.

The Leptospirosis Committee believes that there is neither enough information nor a real need for state-wide control programs at present. Emphasis should be placed on control at the individual herd level.

Anaplasmosis. The test and slaughter plan should be used on a heavily infected ranch, in the heart of the tick country. Vector control measures with insecticides also should be taken. Only by pursuing eradication on such a scale can it be determined whether eradication of anaplasmosis by existing methods is feasible. If not, development of suitable vaccine is imperative.

The Anaplasmosis Committee recommends that the new standardization of the anaplasmosis complement-fixation test be adopted, in order to obtain a uniform test for field diagnosis and control programs, for import and export shipments, and further research studies. Also, it is recommended that funds be appropriated for the continuing production of anaplasmosis complement-fixing antigen and complement.

Summary. Obviously, the foregoing all-too-brief notes are an inadequate report of the work of the U. S. Livestock Sanitary Association. They may serve, however, to indicate the scope of the activities of the association and the important part it plays in protecting the health of the livestock industry.

Each year the proceedings are published, containing in detail all papers presented, committee reports and discussions, together with group actions taken. This 350-page book is distributed only to members, official and individual. Membership is open to veterinarians and lay people in the livestock industry. The annual official proceedings, complete, comprehensive, carefully edited, printed, and bound are worth the membership fee of $5.00 annually. Those interested should write Dr. R. A. Hendershott, Secretary-Treasurer, 33 Oak Lane, Trenton 8, New Jersey.

ABSTRACTS OF PAPERS PRESENTED AT THE SOUTHERN DIVISION MEETING

PEABODY HOTEL, MEMPHIS, TENNESSEE, FEBRUARY 2–4, 1959

Growth of certain lipolytic psychrophilic organisms in pasteurized milk. W. W. OVERCAST AND J. D. SKEAN, University of Tennessee, Knoxville.

Several cultures of psychrophilic microorganisms that were capable of hydrolyzing butterfat on an agar plate at 5° C. were studied, to determine their growth in pasteurized milk and their influence on the flavor under storage conditions. These organisms were classified into three genera: namely, *Pseudomonas, Alcaligenes,* and *Achromobacter.* In addition, one unidentified yeast was studied. Even though all the cultures used in this study were lipolytic at 5° C. on an agar plate, and good growth occurred, the same pattern was not followed when transferred to pasteurized milk. However, those organisms that materially increased in number in the pasteurized milk produced rancid and/or bitter flavors during the storage period.

Along with the cultures isolated from pasteurized market milk, the following species from the American Type Culture Collection were also used: *Pseudomonas fragi* (ATCC 4973), *Pseudomonas fluorescens* (ATCC 11251), *Brevibacterium lipolyticum* (ATCC 11367), *Alcaligenes viscolactis* (ATCC 9036), and *Flavobacterium suaveolens* (ATCC 958). *P. fragi* and *P. fluorescens* grew well in the pasteurized milk and produced rancid and/or bitter flavors. *B. lipolyticum* likewise multiplied, but produced a sour and unclean flavor. *A. viscolactis* and *F. suaveolens* failed to multiply in the milk at the storage temperature.

Evaluation of milk quality from the point of view of the customer. C. B. REEVES, Clemson Agricultural College, Clemson, South Carolina.

A study of more than 500 consumer milk samples shows there is more than an efficient production operation necessary, for a profitable business.

Milk quality means different things to different people. When a chemist thinks of quality, he thinks of composition. When a health officer thinks of quality, he thinks of bacteria. A customer evaluates milk quality principally on flavor. Consumer samples were obtained on the open market. They were analyzed for composition and bacteria, and scored for flavor. Flavor scores and the general attitude of customers generally were in agreement. Specific examples are given:

With an increased interest and discussion of longer shelf life, we find insufficient specific information on milk spoilage at shelf conditions. Many times good-flavored milk could not be so classed when the customer obtained it.

We must furnish to our most sensitive consumers the children, a milk which consistently will be of the best flavor possible.

Preservation of lactic cultures. H. C. OLSON, Oklahoma State University, Stillwater.

Various compounds that are relatively insoluble in water and which can neutralize lactic acid were added to milk cultures of lactic organisms, and the effect of these compounds in preserving the activity of the cultures determined. Calcium carbonate proved superior to the rest of the products tested. Cultures with calcium carbonate added have been held for 8 mo. at 45–50° F. Two of five cultures were as active as they were at the beginning of the trial, two were less active, and the fifth culture was more active. This latter culture was rather slow at the start of the trial, presumably because of bacteriophage infection.

In a preliminary trial, it was found that certain concentrations of glycerol, salt, and sugar aided in preserving the cultures. An experiment was set up in which 10 to 20% glycerol, 2 and 3% salt, 30 and 50% sugar, and calcium carbonate were used alone and in all possible combinations. The cultures were stored at 45–50° F. The cultures were tested weekly, using the activity values on the second transfer as measures of the effectiveness of the various combinations. The results indicate that a combination of 20% glycerol, 3% salt, and 30% sucrose was the most effective, either with or without calcium carbonate added. None of the compounds alone, except calcium carbonate, effectively preserved the activity of the cultures, but several combinations of sucrose with salt or glycerol, or both, maintained the activity of the cultures at fairly satisfactory levels.

Effect of various factors on the activity of frozen starters. J. T. CARDWELL AND J. H. MARTIN, Mississippi State University, State College.

The application of frozen starters in cottage cheese and buttermilk manufacture has not been completely successful. Therefore, a study was undertaken to evaluate various factors which might affect the activity of frozen starters.

The results of this study revealed that the method of thawing the frozen starter was of more importance than the method of freezing (−20 or 0° F.), the incubation time (12–20 hr.), the acidity of the starter to be

frozen (0.73–1.64% acid), the level of solids-not-fat in the starter to be frozen (9–18% S.N.F.), or the age of the frozen starter (0–20 days).

Three methods of thawing were used: (1) slow thaw at 44° F. for 8 hr., (2) fast thaw at 110° F. for 1 hr., and (3) no thaw, transferred directly into the batch starter milk. The slow-thaw and the no-thaw methods were found to be equally effective means of handling the frozen culture, whereas the fast thaw inactivated the starter. Starters treated in this manner developed an average of only 0.215% acidity in 16 hr. at 72° F.; whereas, replicates of the same starter, using the slow- and no-thaw methods averaged 0.896 and 0.898% acidity, respectively.

Factors affecting the spreadability of butter. J. B. MICKLE, Oklahoma State University, Stillwater.

A preliminary series of experiments was conducted to determine the relation between spreadability and hardness of butter. The average correlation coefficient for the two variables in this work was 0.933. In later work, hardness was used as an indication of spreadability. Hardness was defined, for this purpose, as the weight of mercury required to push a sharp metal rod through a ⅜-in.-thick piece of butter.

Milk fat was obtained from melted, fresh sweet-cream butter. After treatment, water and salt were added and the fat was churned into butter again. In Trial 1, the treatment consisted of adding 10% tributyrin or oleic acid to the fat. In Trial 2, the fatty acids in the fat were rearranged on the glycerol molecule by a directed interesterification reaction.

In both trials, the treatment given the fat reduced the hardness of the resulting butter. In Trial 1, when oleic acid was added, correlation coefficients of 0.580 and −0.528 were obtained for hardness vs. per cent fat in the butter, and hardness vs. iodine number, respectively. In Trial 2, a correlation coefficient of −0.675 was obtained between hardness and the amount of liquid fat in the butter.

Rancidity caused by fat shearing in raw milk. A. V. MOORE, Texas Agricultural Experiment Station, College Station.

In an attempt to stimulate turbulence in pipelines, and agitation in bulk tanks, fresh, cooled raw milk from individual cows was stirred to an extent not to materially affect creaming, clustering, or globule size. Fat from samples of milk before and following stirring were titrated for acid value. All except three of 260 tests, made on 48 cows during ten consecutive weeks, showed acid value increases as a result of stirring. Increased acid values were noted on both stirred and nonstirred milks as lactation and the age of cows advanced. Samples observed were from cows 2.5–13 yr. old, varying in stage of lactation from 2 to 22 mo. In nonstirred samples, acid values ranged from 0.21 to 1.79; in stirred samples, from 0.21 to 4.03. At an acid value of 1.00, rancid flavor was definite.

Strontium and calcium metabolism in dairy cows. R. G. CRAGLE AND B. J. DEMOTT, UT-AEC Agricultural Research Laboratory, Oak Ridge, and University of Tennessee, Knoxville.

Experiments were conducted to determine the animal variation in strontium excretion when fed the same ration and the comparative metabolism of strontium and calcium in dairy cows.

From 60 to 85% of the orally ingested Sr^{89} four cows were given simultaneous dosages of Sr^{89} and Ca^{45}. All animals were maintained on a pelleted concentrate ration of 18% protein, with hay as the only roughage. Cows were held four days in digestion stalls prior to a nine-day experimental period. Urine and feces were collected separately. Samples of urine, feces, and blood were taken daily and milk samples were taken twice daily. Standard procedures were used in the preparation of samples for beta counting.

From 0 to 85% of the orally ingested Sr^{89} was found in the feces during the nine-day collection period. The average amounts of Sr^{89} found during the experimental period in the urine and milk, respectively, of eight cows were 1.80 ± 0.77 and 0.55 ± 0.14, expressed as per cent of dosage ± 1 standard deviation. The average ratios of Sr^{89}: Ca^{45} for four cows were 1:1.0 in feces, 1:3.2 in blood, 1:0.4 in urine, and 1:6.2 in milk. The ratios in feces were higher ($P < .01$) during the last four than during the first four days.

Differences between the measured butterfat and the calculated butterfat for a dairy herd. R. W. HENNINGSON, Clemson College, Clemson, South Carolina.

Fat-corrected milk, calculated by the Gaines formula, is the usual method of evaluating milk production in most experimental work. In many cases, a one-day composite sample of milk, collected as part of a herd testing association's program, is the only sample of milk tested for butterfat.

Composite samples of all milk produced by a herd, group of animals, and individual animals for varying periods of time were collected and tested for butterfat content by the Babcock method. The FCM values were computed and compared with the FCM values obtained using the herd testing association butterfat value.

Data from a herd consisting of 200 animals revealed a maximum difference of 0.4% butterfat and 1.5 lb. FCM per animal per day in a

1-mo. period. For the herd, this difference totaled 9,000 lb. FCM and 360 lb. butterfat. Over a 12-mo. period differences totaled 50,400 lb. FCM and 2,016 lb. butterfat.

Influence of a high-fat ration upon the composition of milk and milk fat. I. I. PETERS, R. R. HARRIS, C. A. MULAY, AND F. PINKERTON, Texas Agricultural Experiment Station, College Station.

A continuous winter-feeding trial was conducted involving two groups of ten cows each, five Holstein and five Jersey. The control group was fed a concentrate ration, H, without added fat, whereas the experimental group received concentrate ration, T, containing 6% added stabilized tallow, during the first 6-wk. period, and a similar but less refined feeding fat during a second 7-wk. period. Estimated net energy and protein of the two concentrate rations were the same. Alfalfa hay and sorghum silage were fed ad lib.

Although average daily milk yields with both breeds were higher for Ration T, this difference was not statistically significant. With Holsteins, the per cent fat in milk was statistically significant in favor of Ration H during the second period.

Examination of milk fat (*J. Dairy Sci.*, **39**: 947. 1956) showed that with the Jerseys in Period One the R. I. of the dry fat varied significantly with time, and the alcohol-soluble fraction was significantly greater with Ration T.

The advantage of Ration T fed to Jerseys in Period One over Ration T in Period Two was significant with respect to the R. I. of the alcohol-soluble fat portion.

Comparison of green-chopped Starr Millet and oat silage for lactating dairy cows. L. J. BOYD, Tennessee Agricultural Experiment Station, Knoxville.

Thirteen paired cows (seven Jersey and six Holstein pairs) were used in a reversal-type experiment in which each cow was fed each of the two forages for a 28-day period. The millet was chopped once daily and both forages were fed twice each day to the respective groups. The finely chopped oats, cut in the boot stage, were ensiled by the direct-cut method without a preservative.

Roughage consumption was measured on the group basis. The paired cows were fed the same amount of grain daily and were allowed hay on the group basis at the rate of 5 lb. per cow per day.

During the first period, the average daily production of 4% FCM was 24.3 lb. for the millet-fed group and 23.7 lb. for the cows fed silage. After the cow groups had been switched, the average production was 21.1 and 22.7 lb. for the millet and silage groups, respectively. There were no differences in the butterfat and total solids content of the milk, or in body weight changes, which could be attributed to the type of ration fed.

Throughout the experiment, the cows receiving millet consumed an average of 6.0 lb. more dry matter per cow per day in the form of succulent roughage.

Effect of subnormal growth of dairy heifers upon their first lactation. E. W. SWANSON, A. F. MCFEE, L. M. CARPENTER, AND S. A. HINTON, University of Tennessee, Knoxville.

Six pairs of identical twin heifers were divided at 24 wk. One group was fed a normal ration with no grain after 1 yr. The other group was fed about 70% as much, so that they were 75% of normal weight. After calving at 2 yr., both groups were fed grain and hay alike. Two pairs were lost, due to extreme difficulty of calving of the subnormal heifers. Average weights at 2 yr. were 784 and 594 lb. for animals and subnormals, respectively. All normal heifers started at higher milk yields than their mates. The first 10 wk. average yields of 4% FCM were 21.9 and 18.4 lb. per day for normals and subnormals, respectively. After 10 wk., milk yields of the two groups were more comparable, with the normal group averaging slightly above the subnormal for the rest of the lactation. The normals averaged 4,789 lb. milk, 4.36%, 209 lb. fat in 252 days; and the subnormals averaged 4,468 lb. milk, 4.23%, 189 lb. fat in 254 days. During the first 20 wk. of lactation, the normals ate 3.2% more TDN than the subnormals; thereafter, feed consumption was about equal. During the lactation, the subnormal heifers increased from 75 to 88% of normal body weight.

Effect of Torula yeast on the yield and composition of milk. L. J. BUSH AND E. R. BEROUSEK, Oklahoma State University, Stillwater.

Twelve Holstein cows were used to evaluate the effect of feeding 50 g. of Torula yeast per day on milk production. The cows were paired on the basis of stage of lactation and level of production before assignment to the treatment groups. Grain was fed according to Morrison's standard for cows on pasture, with the amount allotted to each pair being based on their average production. Milk samples were taken for butterfat tests at specific intervals during the 45-day experimental period. No appreciable difference was found between the control and yeast groups with respect either to butterfat test or to yield of 4% fat-corrected milk. Present work on this problem includes a double-reversal trial in which one-half of the cottonseed meal in a normal dairy ration has been replaced by Torula yeast. The effect of the yeast on the proportion of rumen volatile fatty acids also is being studied.

Carotene requirements of dairy cattle. MAGNAR RONNING, E. R. BEROUSEK, W. D. GALLUP, AND J. L. GRIFFITH, Oklahoma State University, Stillwater.

A long time study of carotene requirements of dairy cattle has been reported. Ninety-three animals of the Jersey, Guernsey, and Holstein breeds with 197 gestation periods and 153 lactations were involved in the study. Carotene intakes were varied over a wide range by adjusting the levels of prairie hay which was the sole source of carotene, with the exception of a few Jerseys that received no hay. Cottonseed meal, hominy feed, beet pulp, and cottonseed hulls were used to balance the rations with respect to digestible protein, total digestible nutrients, and fiber.

The carotene intake levels and blood plasma levels of carotene and vitamin A were studied in conjunction with reproduction, lactation, and growth.

It was found that successful reproduction can be expected with Guernsey, Jersey, and Holstein cows when they receive about 75 to 85 γ carotene per pound live weight daily. Normal lactation was observed at carotene levels considerably below those suggested for reproduction. The growth of heifers between the ages of 6 and 15 mo. of age was not affected by increasing carotene intake levels above current recommended growth requirements.

The blood plasma levels of vitamin A and carotene were not reliable indices of the adequacy of carotene intake for reproductive purposes.

Use of commercial cellulase on various plant materials. D. S. RAMSEY, Mississippi State University, State College.

Commercially available "cellulase" enzyme was used to estimate the degree of hydrolysis of various substrates, including two forages. Results with Johnson and Sudan grass collected at varying stages of maturity indicate that more glucose is reelased in early stages than in later stages. It was not possible to estimate cellulose decomposition in forage samples, due to the multiple enzyme nature of the crude product. The enzyme preparation used in this study showed considerable activity on wheat hemicellulose, Johnson grass hollocellulose, carboxymethylcellulose, and cellobiose. A large part of the glucose released by the use of the crude preparation on forages was due to its β-glucosidase activity. In the course of this study it was found that the Nelson-Somoyl method of sugar analysis overestimated the results found with the glucose-oxidase sugar reagent, although the two methods agreed well on pure cellulose. The latter method is to be preferred in work of the type reported here. It was concluded that the crude preparations on the market today are not suitable for the estimation of the susceptibility of cellulose to decomposition.

Comparison of protein supplement and shelled corn for dairy cows on pasture. H. T. BRYANT, R. E. BLASER, AND R. C. HAMMES, JR., Virginia Polytechnic Institute, Blacksburg.

This experiment was carried on for three grazing seasons and was designed to compare the effects of a 16% digestible protein feed and shelled corn on milk production and liveweight on pairs of dairy cows on good pasture. The cows were fed either 1 lb. of protein supplement or ground shelled corn to every pound of 4% FCM, based on milk production during a standardization period. Orchardgrass-ladino clover and orchardgrass-bluegrass-bird's-foot trefoil-white clover pastures, supplemented with alfalfa-orchardgrass and millet during the summer, were grazed in rotation. The eight cows in this experiment were grazed as one herd.

The average milk production of the cows fed the protein supplement was 78% of their production during the standardization period, as compared to 75% for those fed shelled corn. The amount of grain refused in pounds by the cows, ranged from an average of .05 in 1957 to .51 in 1956 of the 16% protein feed, and .03 in 1957 to .54 in 1956 of shelled corn per day. There were no significant differences in weights of cows between treatments.

Optimum replacement rates for effective herd improvement through breeding. Resultant age composition of the herd and milk sales per cow. I. W. RUPEL, Texas A & M College, College Station.

Efforts to improve production in dairy herds by heavy culling and high rates of replacement approach a point of diminishing returns as higher herd average levels are attained. Factors affecting optimum replacement rate include probable or predictable level of producing ability of replacement animals, range or spread in productive capacity of the animals subject to replacement, average age or maturity equivalent of the herd as affected by percentage replacement with young stock, and level of management and herd health that permits retention of known high producers, as opposed to replacement due to disease or breeding failure.

Economic analysis of Virginia D.H.I.A. records for 1957. W. S. GRIFFITH AND K. E. LOOPE, Virginia Polytechnic Institute, Blacksburg.

D.H.I.A. yearly herd summary cards were submitted for a total of 919 dairy herds by Virginia D.H.I.A. supervisors in 1957. Data were reported for each herd on number of cow-days on test, cow-days in milk, total pounds of milk produced, pounds of butterfat produced, value of product, pounds of suc-

culent roughage fed, pounds of dry roughage fed, pounds of concentrates fed, cow days on pasture, cost of pasture, cost of barn-fed roughage, cost of grain, total feed cost, breed of herd, and average weight of herd.

A simple analysis of the data indicates the following: (1) Under-feeding is the number one problem. It is postulated that low production is the result of and not the reason for feeding below the optimum economic level. (2) Present methods of evaluating pasture by difference contain large errors. (3) It does not appear that any of the highest production levels attained are uneconomical. (4) Silage feeding at relatively high levels appears to be one means of lowering feed cost per hundred pounds of milk produced. (5) There is a distinct need for carefully controlled scientific investigations on the feeding of dairy cows, to serve as the basis for extension recommendations.

Measures of persistency of lactation. J. L. FLETCHER, Iberia Livestock Experiment Station, Jeanerette, Louisiana.

Two methods of measuring persistency of milk production were applied to 217 lactations data from 88 Jersey cows. The first method was the ratio of the sum of the absolute deviations of monthly production from the average monthly production to the total 10-mo. production. The second was the ratio of the second 150 days of production to the first 150 days. These methods were applied to the complete milk yield secured from daily milk weights and to single-day milk weights comparable to the data available from weigh-a-day-a-month records. Two sets of single-day milk production data were utilized.

The correlation between the two basic methods in each set of data was near −.88. The correlation between the same method applied to the complete and single-day data was near .95 in all three sets of data.

Production testing for SNF yields of individual cows. N. R. THOMPSON, W. K. STONE, and G. C. GRAF, Virginia Agricultural Experiment Station, Blacksburg.

Accuracy and precision of the lactometric method for estimating TS per cent were investigated. The large and small Watson pattern lactometers gave essentially the same average results, although use of the small Watson model may lead to greater random error, due to interpolation when reading the scale. The lactometric method gave an average per cent TS of 12.85 for eight Holstein-Friesian cows sampled monthly throughout lactation; the gravimetric procedure (A.O.A.C.) gave 12.87. Standard errors of single observations were 0.07 and 0.09, respectively. A lactometer field kit was devised locally, using the USDA pilot model as a pattern and incorporating several improvements. Cylinders of polyethylene plastic for use with the small Watson lactometer were made up on special order. These cylinders are used both as sample containers and as lactometer cylinders in the 102° F. water bath. Monthly sampling of the Virginia Polytechnic Institute herd was begun in August, 1958. The D.H.I.A. supervisor takes somewhat larger samples than usual, and uses them both for lactometer readings and for Babcock tests. Additional D.H.I.A. herds are expected to come into this program of production testing for SNF.

Zinc bacitracin as a silage preservative. L. L. RUSOFF, C. P. BREIDENSTEIN, W. J. MILSTEAD, AND J. E. BERTRAND, Louisiana Agricultural Experiment Station, Baton Rouge.

White Dutch clover was ensiled with various levels of zinc bacitracin (5, 10, and 15 g. bacitracin per ton), molasses (40 and 80 lb. per ton), sodium metabisulfite (8 lb. per ton), and combinations of bacitracin and molasses. The bacitracin was added to the forage in the form of Baciferm 25 (25 g. zinc bacitracin per pound). Ten upright concrete experimental silos (4 by 5 ft.), capacity 1½ tons, were used. With the exception of the control silage, all treated silages had a good odor. Four beef steers, average weight 500 lb., were offered free access to all the silages daily. The position of the different silages in the feed bunks was changed daily. During the 14-day feeding period, the steers consumed almost twice as much silage preserved with 5 g. of zinc bacitracin (⅕ lb. Baciferm 25) per ton as the other silages. The following table gives the results obtained.

TABLE 1

Treatment per ton	pH	Silage consumed per steer
		(lb.)
5 g. zinc bacitracin	4.44	107
80 lb. molasses	3.50	61
10 g. zinc bacitracin	4.30	47
5 g. bacitracin plus 80 lb. molasses	4.28	39
5 g. bacitracin plus 40 lb. molasses	4.80	37
8 lb. Na. metabisulfite	4.33	34
40 lb. molasses	4.35	31
20 g. bacitracin	4.70	28
10 g. bacitracin plus 40 lb. molasses	4.27	16
No preservative (control)	5.07	10

Chemical analyses of the different silages will be presented at the time of the meeting.

Fermentation characteristics of alfalfa silage. Ann R. Stasch and M. E. McCullough, Georgia Agricultural Experiment Station.

Four 20-ton upright silos were filled with bud-stage alfalfa. The forage was direct-cut and preserved as follows: Silo 1, 200 lb. citrus pulp/ton; Silo 2, 80 lb. molasses/ton; Silo 3, 200 lb. ground snap corn/ton; Silo 4, 8 lb. sodium metabisulfite/ton. Samples for analysis were collected with a 2-in. soil-auger through holes in the silo doors. Maximum silage temperatures for Silos 1 through 4 were: 82, 86, 89, and 86° F. The addition of preservatives resulted in only small changes in buffer capacity of the forage. The forage plus preservative had an original dry matter content of 22, 20, 21, and 20%, respectively. Hydrolyzable carbohydrates constituted 17, 18, 19, 37, 26.56, and 13.13% of the dry matter in Silos 1 through 4. Samples were collected four, eight, ten, 12, 16, and 20 days after ensiling for all silos, and at 30, 40, 50, and 60 days for Silos 3 and 4. Observed changes in pH, volatile acids, carbohydrates, and nitrogen indicated that a dynamic fermentation occurred in all silos, with a constant shifting of acid percentages and pH. The fermentation of carbohydrates to acids was linear in Silos 1 and 2, but considerable cell wall or starch breakdown occurred in Silo 3. The fermentation was nearly completed in 20 days in Silos 1 and 2, in about 50 days in Silo 3, and sometime after 60 days in Silo 4. Complete data for each silo will be presented.

Comparison of coastal and common Bermuda hays with oat and alfalfa hays for milk production. W. A. King, C. Conley, and H. J. Webb, Clemson College, Clemson, South Carolina.

Four cuttings of coastal and common Bermuda hays were compared with oat and alfalfa hays in two feeding trials of 112 days each. The alfalfa was U.S. No. 1. The oat was cut in late-milk-to-dough stage the first year and milk stage the second. The fertilizer treatments on the Bermuda hays each year totalled: 300 lb. N, 96 lb. P_2O_5, and 96 lb. K_2O in split applications. The hay yield per acre for the coastal was 4.16 and 6.70 tons and, for the common, 1.96 and 3.25 tons.

The hay consumption by years averaged: alfalfa—22.7 and 22.5 lb., oat—11.4 and 17.7 lb., coastal—20.4 and 19.7 lb., and common—21.4 and 20.6 lb. Thirty pounds corn silage per head was fed daily, as well as concentrates. The daily 4% milk production by years averaged: alfalfa—31.2 and 30.3 lb., oat—27.7 and 26.5 lb., coastal—27.4 and 25.2 lb., and common—30.0 and 28.0 lb. The per cent of expected milk production was: alfalfa—96.9 and 98.2%, oat—86.8 and 85.9%, coastal—88.4 and 81.6%, and common—89.9 and 91.2%. Over-all for the two years oat hay in the milk stage was worth 88%, coastal 87%, and common Bermuda hay 93%, of the alfalfa hay.

Effect of preservatives on the silo losses and feeding value of alfalfa silage. L. R. Sisk, M. E. McCullough, and O. E. Sell, Georgia Agricultural Experiment Station, Athens.

A study was made of the effect of preservatives on silage consumption, animal performance, and silo losses and temperatures with a first-cutting, bud-stage alfalfa ensiled in four upright silos. Preservatives used per ton of green forage were: citrus pulp, 200 lb., molasses, 80 lb., ground snap corn, 200 lb., and sodium metabisulfite, 8 lb. in Silos 1, 2, 3, and 4. The silages were fed as the only roughage to cows grouped according to level of production and body weight. Average daily intakes of the silages were 124.6, 116, 84.2, and 72.6 lb. for Silos 1 through 4. The 28-day changes in FCM were +9, −15, and −2% for Silos 1, 2, and 3, respectively. The metabisulfite silage in Silo 4 was unsatisfactory for milking cows. Dry matter losses in the silos were significantly higher from the molasses and metabisulfite silages: 24.5% and 38.2% as compared to 13.3% and 11.4% from the citrus and snap corn preserved silages. The sources of these DM losses were as follows: 29, 25, 28, and 7% in the seepage; 71, 75, 72, and 92% as fermentation loss in Silos 1 through 4. Maximum silo temperatures were 82, 86, 89, and 86° F.

Value of Tracy sorghum silage cut at two stages of maturity for feeding producing dairy cows. R. E. Leighton and I. W. Rupel, Texas Agricultural Experiment Station, College Station.

On a double-reversal feeding experiment, 20 dairy cows produced 426 lb. more milk when they were fed Tracy sorghum silage, harvested when the oldest seed was in the soft-dough stage, than was produced when the animals were fed the same amount of silage harvested when the same crop was in the hard-seed stage. At $5.80 per hundred pounds, this additional milk was worth $24.71, making the less mature silage worth $1.60 more per ton when fed to producing dairy cows.

On the basis of digestion trials conducted during the experiment, the immature sorghum silage contained 23.5% and the mature silage 18.5% total digestible nutrients. There was little difference in the percentage of dry matter in the two silages and almost no difference in the acidity or carotene content. Measurements of the yield per acre were not accurate enough to reveal any difference, but on the basis of this experiment, the mature silage would have to produce 19 tons per acre if the immature silage produced 16 tons, in order to yield equal amounts of total digestible nutrients per acre.

Free choice supplementation of corn and sorghum silage rations fed to growing dairy heifers with a urea-alcohol-molasses preparation. KENNETH BIERWIRTH AND O. H. HORTON, University of Arkansas, Fayetteville.

A feeding trial of 120 days' duration, using 20 pure bred heifers owned by the University of Arkansas, was conducted to determine the feasibility of feeding a urea-alcohol molasses preparation free-choice, in comparison to cottonseed oil meal, as the source of protein or protein-equivalent supplement. The heifers were divided into four groups of five animals each. Group I receiver corn silage and the urea-molasses mixture ad libitium. Group II received corn silage and enough cottonseed oil meal to balance the daily requirement of protein as shown by the N.R.C. requirements. Groups III and IV were fed in a similar fashion, except that sorghum silage was substituted for the corn silage. Ground corn was used to supply the additional TDN required by each animal to meet the N.R.C. requirements for energy.

Weight gains were 1.00, 1.22, 0.97, and 1.39 lb. per day for Groups I, II, III, and IV, respectively. The pounds of protein equivalents per pound of grain were 1.81,* 1.21, 1.85,** and 1.11 for Groups I, II, III, and IV, respectively. There appeared to be no difference between the kind of silages fed.

* Statistically significant at the .05 level.
** Statistically significant at the .01 level.

Herd averages in sire evaluation. V. L. BALDWIN, Virginia Polytechnic Institute, Blacksburg.

Bull proofs or daughter averages fail to predict performance in high-producing herds.

Matching each record with its comparable herd average, and comparing the two accumulated averages, can help indicate the sire's performance in known environment levels.

Grouping herd average levels and comparing with daughters, records can help predict performance in individual herds.

Sire 860768 had 279 daughters with 417 D.H.I.A. records and accompanying herd averages in 133 Virginia herds. In seven herd average groups separated in 50-lb. increments, the sire's daughters produced higher than each composite average from 231 to 518 lb. butterfat in a year.

In this sire's several D.H.I.A. proofs, the daughters averaged about 440 lb. butterfat compared to about 425 lb. for the dams. Likewise, 320 daughters with 529 records in five states averaged 441 lb. butterfat and 12,099 lb. milk.

Herd owners with higher herd or individual cow averages than the proof hesitated to use this sire. When his performance at each environment level was known, the sire could be used with reasonable confidence.

With a few herds, certain influences may bias the herd average as an environment measure, unless carefully used.

Effect of air conditioning and other cooling practices on physiological responses, semen production, and fertility of bulls under southern conditions. T. E. PATRICK, H. C. KELLGREN, P. E. JOHNSTON, GEORGE HINDERY, J. O. SHELWICK, AND JANE BANKSTON, Louisiana State University, Baton Rouge.

Eighteen mature dairy sires, nine Jersey, six Holstein, and three Guernsey, were randomly assigned by breeds to three groups. Group 1 was kept in a concrete block barn equipped with ten ceiling fans and two exhaust fans. In addition to the air movement, the animals were exposed to a fine mist water spray. Group 2 was kept in one-acre pastures with aluminum covered sheds and tree shade. Group 3 was kept in a temperature control chamber which was maintained at about 65° C. and in a humidity of 12 mm/Hg vapor pressure. The experiment began April 1 and ended November 15, 1958. Body temperatures for all three groups of bulls showed slight declines during the experimental period. The respiration rates for the pasture group increased during July and August. Respiration rates remained constant for the other two groups throughout the study. Protein bound iodine declined sharply in June for all groups and remained at a relatively low level through October. Semen quality remained at a high level for all groups throughout the experiment. Slight declines in per cent progressive motile spermatozoa and moderate increases in per cent abnormal spermatozoa were observed for all groups.

Effect of intrauterine antibiotic treatment on low fertility dairy cows. S. D. MUSGRAVE, W. N. PHILPOT, AND E. W. JONES. Oklahoma Agricultural Experiment Station, Stillwater.

This experiment was conducted to evaluate the efficiency of streptomycin and penicillin as a means of controlling uterine infections, including *Vibrio fetus*, that might reduce fertility. The test of effectiveness was reproductive behavior. A dairy herd was divided into three groups for this purpose. Group I cows served as controls and were not treated. Group II cows were given a single intrauterine injection of 1 g. of streptomycin and one million units of penicillin in 15 ml. of double-distilled water, at each estrus following parturition, until the cow was bred at first service or at 30 and 50 days postpartum. Group III cows were treated as Group II, but treatment was continued until conception occurred or the experiment ended. All cows were artificially inseminated with antibiotic-treated semen. There were 55, 49, and 46 cows involving 203, 220, and 197 estruses per group, respectively. Treatment of Groups II and III did not reduce

services per conception. Treatment was favorably associated with incidence of normal estrus cycle length and frequency of normal estrus cycles following infertile service.

Intensity of estrus in dairy cattle in Louisiana. J. G. HALL, C. BRANTON, AND E. J. STONE, Louisiana State University, Baton Rouge.

Intensity and duration of estrus were determined on all cows calving in 1957 and on heifers being bred for the first time this same year in the University herd. A total of 173 females (99 Holsteins and 74 Jerseys) was involved. Intensity of heat was scored as follows: 3 = standing and very active heat; 2 = moderately active heat, but nonstanding, and 1 = very mild heat. Both intensity and duration of estrus were observed at 6-hr. intervals throughout each day of the year, excepting during four ten-day periods when they were observed at 2-hr. intervals. Data on the Holsteins have been analyzed partially, and those on Jerseys will be analyzed soon.

There were 308 estrual cycles observed on the 99 Holsteins. The average duration of heat was 10.9 hr. Parous females averaged 10.1 hr. as compared with 13.2 hr. for nonparous females. The average intensities of estrus for the parous and nonparous females were 2.81 and 2.74, respectively. There was an inverse relationship between average intensity of estrus and duration of estrus, i.e., the higher the average intensity of estrus the shorter was the duration of heat. Little or no relationship was observed between intensity of estrus and fertility.

In vivo studies on the deamination of amino acids by rumen microorganisms. C. G. LOOPER, O. T. STALLCUP, AND F. E. REED, Arkansas Agricultural Experiment Station, Fayetteville.

Several amino acids, β-alanine, DL-aspartic acid, glycine, L-glutamic acid, and L-lysine, were fed on an equal-nitrogen basis to a fistulated steer. The basal diet consisted of cottonseed hulls and blackstrap molasses. Each amino acid was studied individually and was added to the basal ration for three days, with samples being taken on the third day at hourly intervals. Determinations of total nitrogen, protein nitrogen, and ammonia nitrogen were made on each sample. Nonprotein nitrogen was calculated as the difference between total nitrogen and protein nitrogen.

The rate of ammonia production was significant at the 1% level from DL-aspartic acid and L-glutamic acid, whereas that from β-alanine was significant at the 5% level. Ammonia produced from L-lysine and glycine was not significantly different from that produced on the cottonseed hull–molasses diet.

Effect of various diets on the rumen development of dairy calves. W. C. LOE, O. T. STALLCUP, AND H. W. COLVIN, JR., Arkansas Agricultural Experiment Station, Fayetteville.

Sixteen newborn Holstein bull calves were divided into four groups of four calves each. Group 1 was fed milk, plus a mineral mix; Group 2, fatty acids plus milk, plus mineral mix; Group 3, hay, grain, and milk; Group 4, milk, plus mineral mix, plus rumen liquor from a fistulated steer on a diet of alfalfa hay. The average volumes of the stomach compartments, determined 13 wk. after beginning the experiment, were as follows: The reticulo-rumen, 4,838, 4,362, 8,710, and 3,815 ml. for Groups 1, 2, 3, and 4, respectively. The omasum, 104, 115, 442, and 84 ml. for Groups 1, 2, 3, and 4, respectively. The abomasum, 1,004, 1,691, 1,507, and 645 ml. for Groups 1, 2, 3, and 4, respectively. The average moisture-free weights were: reticulo-rumen, Group 1, 202 g.; Group 2, 263 g.; Group 3, 504 g., and Group 4, 140 g. The omasum, 43, 49, 125, and 28 g. for Groups 1, 2, 3, and 4, respectively. The abomasum, 119, 143, 121, and 109 g. for Groups 1, 2, 3, and 4, respectively.

Effect of height of shade on solar radiation under Louisiana summer conditions. J. E. JOHNSTON, F. T. WRATTEN, F. B. HAMBLIN, AND A. GUIDRY, Louisiana State University, Baton Rouge.

An experiment was set up to evaluate the effect of height of shade on solar radiation receipt in the humid Gulf Coast area during July and August. Three experimental shades 6 by 12 by 10 ft. high were erected in an open pasture. The shades were covered with a laminated plastic, white on top and black on the underside (Bakelite). Radiation receipt was estimated using three "Beckman and Whitley" total hemispherical radiometers suspended at varying distances from the roof in the center of the shade. As the distance from the roof to the radiometer was increased, the radiation receipt also tended to increase, indicating that the effective temperature of the shade material was lower than that of the sky. This was probably due to the reflection of solar radiation from the numerous white cumulus clouds usually observed at this time of year. On the basis of this study, it is recommended that shade height not exceed 6–8 ft. in the Gulf Coast area. Further data on radiation intensities will be reported.

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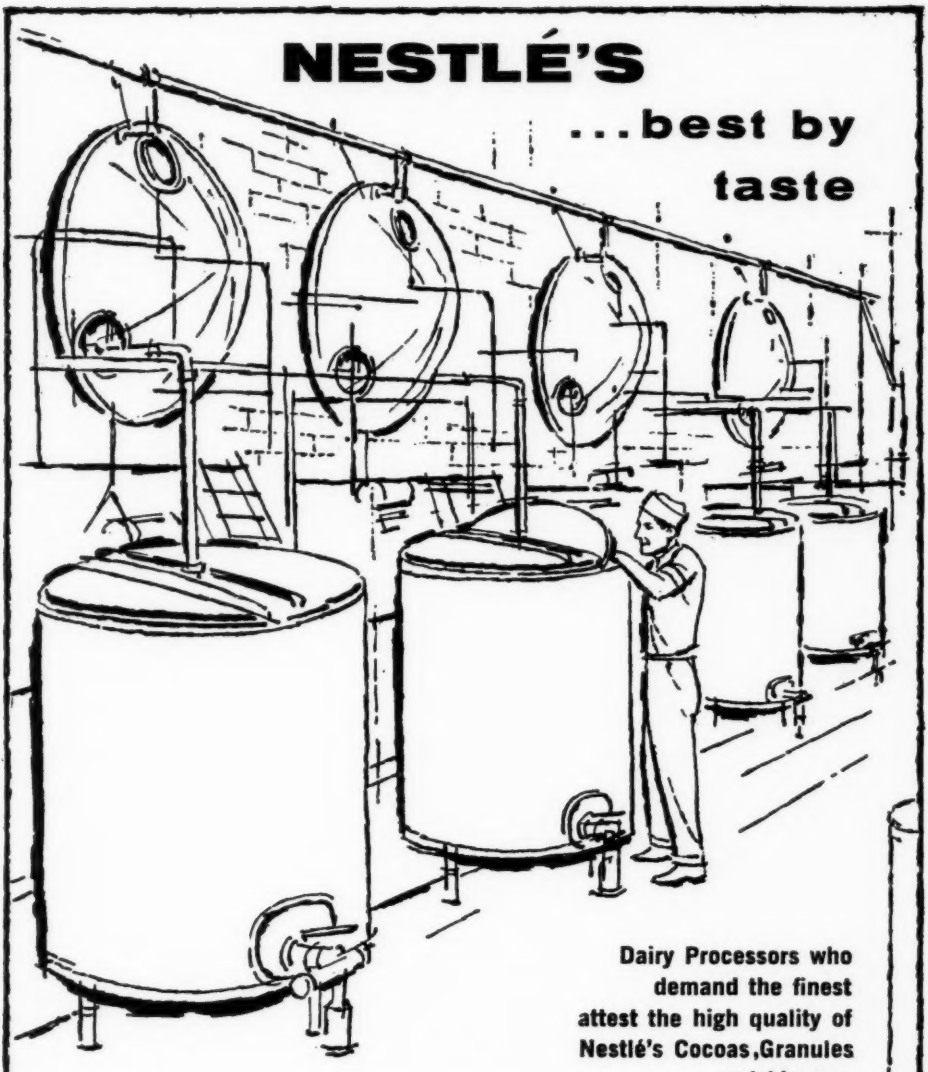

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